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THE BULLETIN OF THE ASSOCIATION
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ENDOCRINOLOGY

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MODIFICATIONS OF STRUCTURE IN THE PANCREAS OF MICE INOCULATED WITH ADENOCARCINOMA

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PAVIA, ITALY

Glycogen has been known as a product of cellular activity of tumors ever since the first investigations of Chambard, which were confirmed by other observers; among these were Cornil and Ranvier, Langhans, Marchand, Beaumann, Lubarsch, Béhr, Best, Borst, Pentagna, Freund, Révész, Cori, Tesauro, Russel and Borghi.

Rondoni in 1912 demonstrated an increased rate of growth in tumors of rats on a diet rich in carbohydrates. Braunstein, Fulci, Haaland, Beed, Woglon, Handel, Tademura, Cramer, Pentimalli and Baker confirmed these observations.

The merit of placing these studies on a firm basis belongs to Warburg and his school. Sections of tumors in Ringer's solution, to which glucose was added, showed a diminution in pH of the liquid with production of lactic acid, after a few hours in the incubator; a glycolytic activity of the tumor sixty times greater than that of normal tissues was demonstrated.

Silberstein, Freund, Waterman, Borghi and, in our school, Piccaluga and Cioffari examined the action of insulin on the growth and evolution of tumors and found a delay in growth but no regression after inoculation.

The pancreas, which plays such an important rôle in the regulation of carbohydrates in the blood, should show the effects of such glycolytic activity. A study of the changes in the structure of the pancreas, particularly in the insular portion, in relation to inoculated tumors was therefore undertaken.

EXPERIMENTS

The animal chosen for the experiments was the small white rat inoculated with adenocarcinoma, of the Ehrlich type; this gives a growth of 90 per cent, develops within twenty days and is fatal in about 40 days. Eighteen to twenty days after inoculation the animals were sacrificed, the pancreas was removed and the tail, body, and head were examined. As fixatives the following were used: alcohol, formalin and Zenker's fluid. The following stains were employed: haematoxylin (Carazzi or Heiden-

hain), eosin, orange G, Congo red and Pappenheim's mixture modified by Stropeni. The last is the most useful for studying the intimate structure of the pancreas, for it differentiates zymogenous from the insular tissue, and it shows the distinction in the islets between chromophil and chromophobe cells. Langerhans islets were counted according to the Laguesse-Abbruzzese method and Nakamura's technique.

Sections 5 microns in thickness were made and ten preparations of each portion of the pancreas were examined. A small piece of paper with perforations one millimeter in diameter was placed on a piece of glass, under an objective and the islets were counted. The total number divided by ten gives the relative average for every portion. In order to determine the modifications in the pancreas caused by the tumor the pancreas of normal rats of corresponding age and weight was studied.

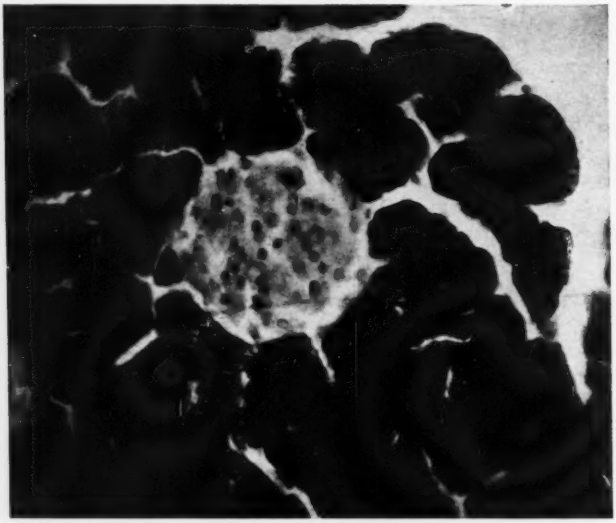

Fig. 1. Section of head of normal pancreas. Fixed in alcohol, stained with Heidenhain hemotoxylin and orange G, K. 2/8. Enlarged 390 diameters.

The same method of fixation, staining, and counting of the islets was used for the pancreas of the control animals which were kept on the same diet as the experimental animals. The pancreas of rats is placed in a fold of peritoneum, from the hilum of the spleen to the stomach and duodenum; as in most mammalia it is possible to distinguish the splenic portion or tail, the body, and head.

Histologically it is composed of two principal tissues, zymogenous and insular. The former consists of acini of polyhedral cells in contact with each other so as to limit the lumen of the acinus, where the centro-acinose cell may sometimes be observed. The cells have a large nucleus placed at periphery during the digestive phase, and by suitable methods

of staining granules of zymogen can be readily distinguished. Even with the usual stains Langerhans islets are in evident contrast to the zymogenous tissue; they are round, or oval, and of various sizes (Fig. 1). They are irregularly placed throughout a section, stain more clearly than the secretory acini and are devoid of granules of zymogen characteristic of the latter. The cells of the islets are smaller than those of secretory acini. Two types are distinguished, chromophil cells and chromophobe cells. The former are of lymphocytoid type with small nucleus, round, intensely colored and rich in chromatin; the latter clearer, with strongly acidophil protoplasm, and quite twice as numerous as the former. Islets are larger and more numerous towards the splenic portion, smaller and rarer towards the body and head.

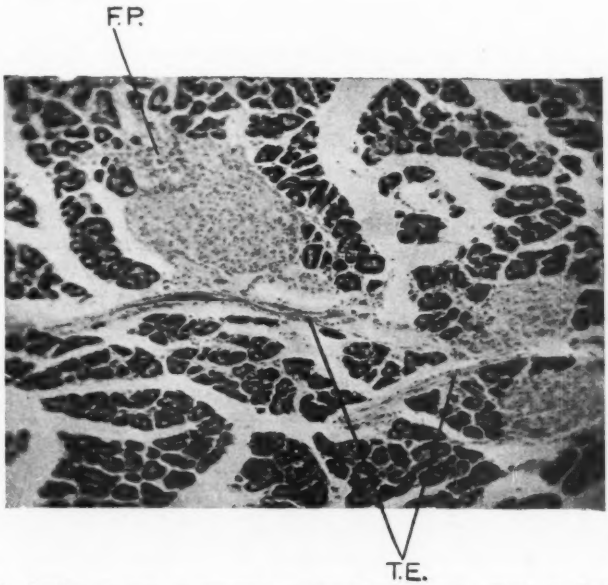

Fig. 2. Section of tail of pancreas of tumor-bearing rat. Fixed in Zenker's fluid, stained by Stropeni's method. K. 2/3, 1.130. F. P. Transition form. T. E. Excretory duct.

Counting shows an average of 2.2 for the tail, 1.5 for the body, 1.1 for the head. The diameter of the islets variates from 40 to 300 microns.

The pancreases of animals with tumors show no macroscopic change worthy of note.

On microscopic examination the pancreatic acini which in normal subjects reach a diameter of about 40 to 60 microns, are from 30 to 45 microns in the inoculated rats; cells which have a medium height of 15 in normal animals are reduced to 13 in the cancer-bearing subjects (Fig. 4). The whole microscopic field is different from that of normal animals; the acini appear less compact and limited by interstitial connective tissue (Fig. 2).

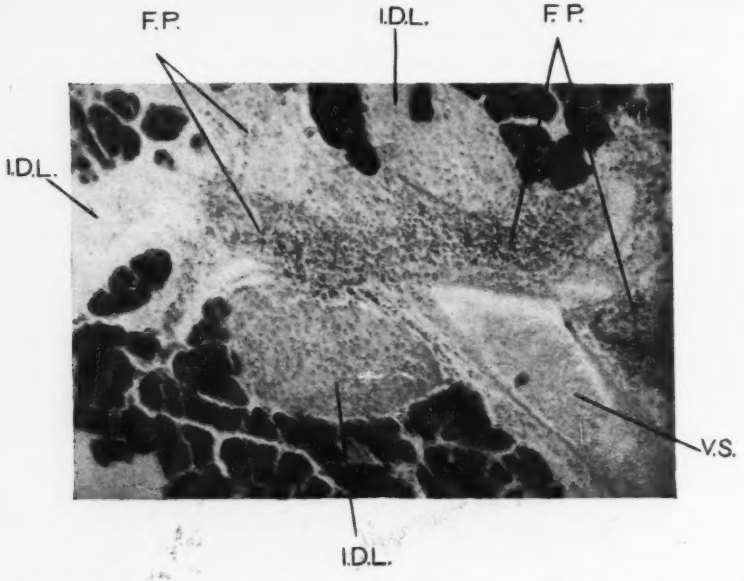

Fig. 3. Section of body of pancreas of tumor-bearing rat. Fixed in formalin, stained by Stropeni's method, K. 2/5. Enlarged 200 diameters. I. D. L. Islands of Langerhans. F. P. Transition form. V. S. Blood vessel.

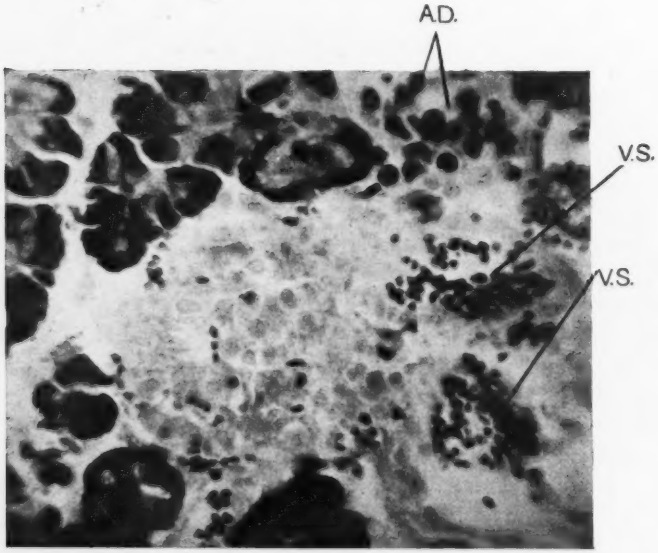

Fig. 4. Section of body of pancreas of tumor-bearing rat. Fixed in alcohol, stained by Stropeni's method, K. 2 immersion. Enlarged 270 diameters. A. D. Acini in process of formation of transition forms. V. S. Blood vessel.

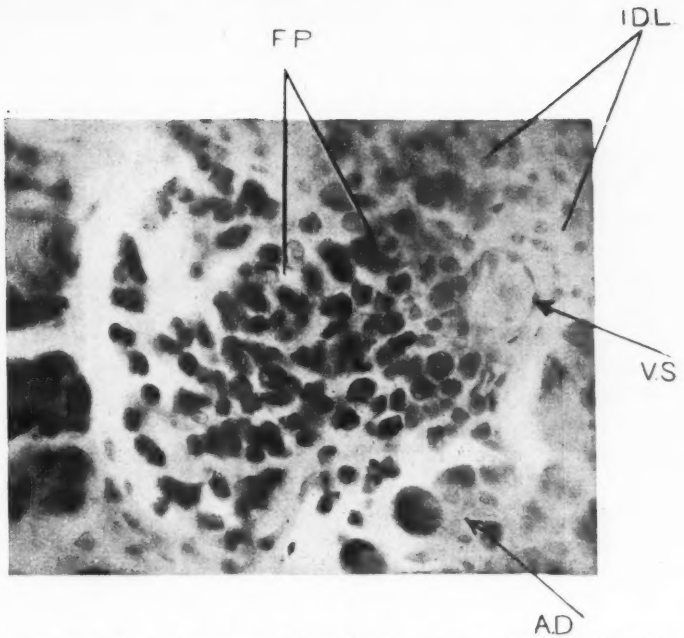

Fig. 5. Section of head of pancreas of tumor-bearing rat. Fixed in formalin, stained by Stropeni's method, K. 2 immersion. I. D. L. Islands of Langerhans. F. P. Transition form. A. D. Acini on way to separation. V. S. Blood vessel.

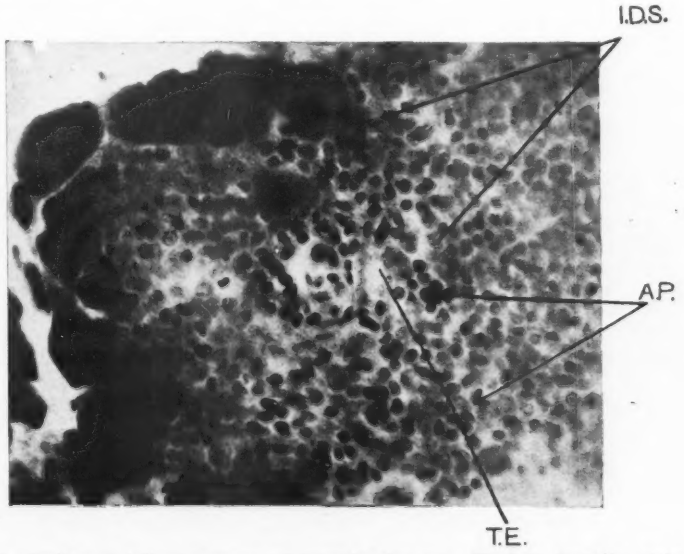

Fig. 6. Section of tail of pancreas of tumor-bearing rat. Fixed in formalin, stained by Stropeni's method, K. 2 immersion. Enlarged to 570 diameters. Group of special transition forms in close relation with old islands of Langerhans. I. D. S. with excretory duct. E. D. interpolated in a cellular mass in which pancreatic acinar cells A. P. may be distinguished.

The changes in the zymogenous tissue are not as marked as those in the islets of Langerhans, which appear remarkably increased in volume; while the maximum diameter of islets in normal young rats is about 200-300, in the inoculated ones it may reach 750 microns (Fig. 3). A more accurate study of the constituent elements shows that while in the former the diameter of the cells of the islets reaches up to about 8.5 microns, in affected animals they may reach up to 12 microns in diameter. The main histological changes are observed in the islets. In normal rats they are nearly all limited by a well distinguished capsule; but in animals affected with adrenocarcinoma a continuity of zymogenous tissue with the insular tissue is noted in some places.

On account of the disappearance of that limit between endocrinous and exocrinous tissue the islets tend to become irregular; cellular strands penetrate amongst the pancreatic acini which lose their characteristic alveolar shape. They show cells reduced in volume; they are no longer polyhedral but almost round like the chromophil insular cells (Figs. 2, 3, 4, 5, 6).

The diameter of these special forms of transition cells is intermediate between that of zymogenous and the insular cells, their average being about 10 microns. In preparations stained by the Stropeni method, or by Heidenhain's haematoxylin, the granules of zymogen become quite evident; they appear less numerous and smaller than in insular cells.

The increase in insular tissue must depend either on the insular cells, which appear enlarged about twenty-five per cent in volume, or mainly on the addition of special transient cells around the old islets that account for about seventy-five per cent of the increase.

The following table shows the different portions of the pancreas examined both in normal and in inoculated animals. It shows that there is also a slight numerical increase in addition to the increase in size.

Normal Pancreas	Number of the Islets of Langerhans			Pancreas of Tumour Bearing Animals	Number of the Islets of Langerhans		
	Head	Body	Tail		Head	Body	Tail
1	1.2	1.4	2.3	1	1.5	1.8	2.6
2	1.3	1.1	2.6	2	1.7	2.	2.5
3	1.1	1.3	2.1	3	2.	1.3	3.3
4	0.9	1.6	1.9	4	1.2	1.7	2.7
5	0.8	1.5	2.3	5	0.8	2.1	2.1
6	1.4	1.5	2.6	6	1.4	1.6	2.5
7	1.2	1.8	2.2	7	2.	1.7	2.2
8	1.2	1.5	2.7	8	1.3	1.5	1.9
9	0.8	2.	2.1	9	1.3	2.1	3.
10	1.2	1.3	1.8	10	1.5	2.2	2.8
Average.....	1.11	1.50	2.26	Average.....	1.47	1.80	2.28
	General average = 1.62				General average = 1.85		

DISCUSSION

To facilitate the interpretation of the changes described above it is necessary to review modern ideas on the genesis of the islands of Langer-

hans, for since the classical experiment of v. Mering and Minkowski in 1890 (two years after the work of Martinotti in Italy) demonstrated that the removal of the whole pancreas in dogs was followed by a fatal diabetes, interest was focused on the islets of Langerhans which were now considered to constitute an organ of internal secretion for regulating carbohydrate metabolism. Researches on the fine structure of the islets of Langerhans followed with the object of determining how they conducted themselves in diabetes and in other pathologic processes. The results obtained were not uniform; the work of Diekoff, Kashara, Opie, Weichselbaum and Kyrle, Curtis and Gellè, Lepine, Lanceraux, Thomas and Delamare, Visentini, etc., supports the view that the islets of Langerhans are important in the pathogenesis of diabetes mellitus, while, on the contrary, Hansemann, Gutmann, Karakascheff, Beitmann and Pende oppose the theory and, on the other hand, Herscheimer, Tammovisky, Lino, Fahr, etc., consider diabetes as a disease of the whole pancreas, including islets and glandular acini.

The pathological processes which occur in the pancreas in different diseases are pertinent to this problem; many authors have published cases in which the atrophic process affected only the glandular parenchyma while insular tissue was not affected. Observations of this type support the view of A. A. Ssobolew, Koch, Lang, etc., on the independence of the islets of Langerhans. But Pepere on theoretical grounds contended, "That atrophic-necrotic and sclerotic processes which may develop in the acinus parenchyma and in its supporting stroma must necessarily propagate themselves by repercussion in part on a certain number of islets which are so closely and intimately bound with the acini." In 29 cases of diabetes mellitus Fahr was able to demonstrate the transformation of the glandular parenchyma into islets, and the studies of Lavowsky, Saguki, Seyfarth, Tammowisky and others have added to this knowledge which shows change of exocrine tissue into endocrine. Singular transition forms originating from the acini of zymogenous tissue were described. In a case of congenital syphilis Stoerk found the islets normal. De Vecchi found that the acini in the pancreas of syphilities are modified as a result of proliferation and become very like the islets of Langerhans. Lino after examining a considerable number of specimens concluded that in particular conditions of the organ associated with the gradual and slow suppression of pancreatic parenchyma acini may be transformed into islets.

The histological changes in the pancreas in conditions of vitamin deficiency (scurvy) throw some light on this question. Rondoni, Montagnani, Monastero and Borghi have shown a remarkable abundance of the islets of Langerhans which were increased in number and also in volume in animals subjected to a scorbutic diet. These investigations recall the hypotheses put forward on the ground of anatomical and physiopathological facts by Laguesse, who for the first time in 1893 established the functional significance of the islets postulating a sort of functional cycle

in which the islets might change into parenchyma cells and vice versa, assuming at various times now endocrine and now exocrine functions.

The founder of the "Theorie de balancements" and his school showed the genesis of special cells in glandular tubuli, which separated and formed cellular masses which they considered to be islets of Langerhans. Later these islets might be transformed again into simple glandular tubuli with ducts originating from the central cells while the peripheral cells became glandular.

While a number of observers supported Laguesse, *e.g.*, Dale, Clara, Seifarth, etc., who found an increase in the substance of the islets after stimulating with secretin, others, *e.g.*, Lowenfeld and Jaffé, after stimulating with injections of atropine, Else and Wathermann with adrenalin and Lazarus and Tiberti with adrenalin and phlorizin found no such modification.

More interesting for this problem are the researches of Ssobolew, Diamare and Marassini which deal with a hyperfunction of the islets of Langerhans in animals treated with glucose over a long period; of Dale who described an acino-insular transformation after feeding on diet rich in glucose, and of Borghi and Muggia who have noted morphological modifications of islets of Langerhans with remarkable increase in size, describing even some carcinoid changes in the insular cells, in animals treated by glucose. There is no uniformity in the observation on the after effects of ligature of the pancreatic ducts.

While an atrophy of glandular parenchyma and hypertrophy of the islets of Langerhans is described by every one, the possibility of insular tissue increasing at the expense of zymogenous tissue is still debatable (Schulze, Laguesse, Pende, Lombroso, Tschassownikows, etc.). After thyroidectomy (v. Noorden) and ovariectomy (Rebaldi) there is an increase of endocrine tissue, while there are no changes after splenectomy (Fichera). The experiments on fasting animals by Statkewitch, Dale, Vincent, Thompson, Hansemann, de-Gaetani, etc., who find an increase of insular tissue and explain it as obtained by a transformation of acinous tissue, has not been confirmed by Jarotsky, Sculze, Lane, Bensley, Homans, etc.

Embryological studies have shown that the islets of Langerhans originate from ducts of glands; Pearce and Weichselbaum and Kyrle are certain that the insular tissue and the zymogenous tissue of mammalia have a common embryological origin from a cellular epithelial cord. Moreover, these observations have been confirmed by Laguesse, who established that glandular parenchyma and the islets are of epithelial origin, and Kyrle observed after transplantation of small pieces of panereas into the spleen that the generative processes which follow show that islets and zymogenous tissue originate from the epithelium of the excretory ducts.

A certain connection between islets and acini has been described by Nakamura in foetuses. This observer found that the development of insular cells is closely related to that of acinous cells from the sides of the

ducts, while Hickel and Nordmann admit that islets of Langerhans are formed in man all his life at the expense of the secretory system. According to these observations the centro-acinous cell has the power to proliferate and to assume the character of insular cells. The genesis of the islets is a difficult problem and various authors working with different techniques and studying the pancreas in the first embryological and in different physiopathologic stages put forward new views, but the problem is not yet solved. The extensive literature on the subject may suggest that insulo-acinous transformations are interpretations of microscopic findings rather than objective facts.

The foregoing statements have a bearing on the changes in the pancreas induced by tumors. Pre-eminently two changes stand out: (1) a remarkable increase in the volume of the islets, which may be doubled; (2) an increase in number of the islets of Langerhans in the different portions examined. These facts have been established by measurements of islets and by counting. It remains to be determined whether this increase in the volume of the islets is due to hypertrophy of each element, to cell division, or to transformation of elements of zymogenous tissue.

A slight hypertrophy is found; the volume of insular cells may increase by 25 per cent. It is not difficult to find rather rare karyokinetic figures in insular tissue. There is an undoubted separation of acini with reduction in volume of elements which constitute them, the loss of their granules of zymogen and their transformation into cells similar to the chromophil cells of insular tissue, changes which are clearly seen at the periphery of the old islets. All these factors play a part.

The importance of these changes in relation to the problem of tumors has to be considered. The glycolytic activity of blastomata and the hyperglycaemia and diabetic state in subjects having tumors indicate that the pancreas undergoes changes in the islets of Langerhans which up to a certain point control the increase of glucose in the circulation. The pancreas of animals with tumors undergoes changes chiefly in the islets of Langerhans which by hypertrophy and hyperplasia regulate the glucose in the circulation.

SUMMARY

1. The pancreas of small rats inoculated with tumors shows changes chiefly in the islands of Langerhans.

2. These changes consist of hyperplasia which causes an increase in the volume which may be doubled and an increase in number of the islets of Langerhans in different parts of the pancreas.

3. Increase both in volume and in number are accounted for by transformation of zymogenous into insular tissue, by hypertrophy of the persistent elements and by some cell division.

4. These alterations are interpreted as compensatory for the purpose of regulation of glucose which is increased in tumor-bearing animals.

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MIGRAINE

A STUDY BASED ON ONE HUNDRED CASES

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Believing that a statistical study might be of value in determining the etiology of migraine, one hundred cases were studied. These individuals gave the usual history of headache, nausea, vomiting, etc., incident to this disease. Hemicrania was a frequent complaint and not infrequently abortive attacks were present. Practically every one of the subjects had a definite enlargement of the thyroid gland, several having been operated upon for goiter. Full cognizance is taken of the fact that this report originates in a goiter belt and this may account for the high incidence of thyroid enlargement as well as the fact that there were twice as many women in this series as men.

As for many other diseases, an endocrine etiology has been suggested a great many times. This study provides a more rational basis for speculation as to the etiology of this clinical entity.

Heredity: A family history of migraine in one or more members of the immediate family was obtained in 39 per cent of the cases. A family history of goiter was obtained in 13 per cent.

Sex: Of the one hundred cases, 69 per cent were female and 31 per cent were male.

Age: The ages of the females ranged from nine years to seventy-two years, the average age being 36.8 years. The ages of the males ranged from sixteen years to sixty-two years, the average age being 37.9 years.

Duration: Concerning the duration of the migraine attacks, more or less definite statements were obtained from fifty-one patients. The duration of the attacks ranged from six months to forty-one years, the average being 8.35 years. Many stated the duration as "all my life," "since childhood," etc., so that no definite statement can be made concerning these.

Weight: The weight of the females ranged from 56 pounds (nine years old) to 226 pounds, the average being 140 pounds. The weight of the males ranged from 124 pounds to 190 pounds, the average being 160 pounds.

Height: The height of the females ranged from 34½ inches (nine years old) to 69 inches, the average being 64.6 inches. Excluding the nine-year-old girl, the range is from 58½ inches. The height of the males ranged from 63 inches to 75 inches, the average being 70.4 inches. Particular attention was paid to the heights of the immediate families. Of the 69 females, a family history in regard to height was obtained in 51.

Of the 51 females, 36 stated that one or more of the immediate family (father, mother, brother or sister) was either 72 inches tall or very close to this height. This gives a percentage of 70 in whom one member of the immediate family was either 72 inches tall or close to this height; none less than 71 inches. In the 31 males, an immediately family history in regard to height was obtained in 21. Of these 21, 17 or 80 per cent stated that some member of the immediate family was either 72 inches tall or very close to 72 inches, none less than 70½ inches. In both female and male, usually more than one member approached this height. These facts are of importance with reference to the pituitary relationship to skeletal development and the origin of migraine.

Blood Pressure: The systolic blood pressure of the females ranged from 90 to 190 mm. mercury, the average being 129 mm. The diastolic pressure ranged from 48 to 140 mm., the average being 80 mm. The systolic blood pressure of the males ranged from 90 to 210 mm., the average being 128 mm. The diastolic pressure ranged from 60 to 130 mm., the average being 80 mm.

Basal Metabolism: Basal metabolic estimations of the females were made in 51 cases of the 69. Of these 51 cases, 21 or 40 per cent showed a plus rate, ranging from plus 1 to 22 per cent. The average increase was 9 per cent. Thirty cases or 60 per cent showed a minus rate ranging from 1 to 23 per cent. The average was 10.8 per cent. Basal metabolic estimations of the males were made in 23 cases of the 31. Of this number 6 or 26 plus per cent showed an increased rate, ranging from plus 4 to 30 per cent, the average being 17.5 per cent. Seventeen cases or 73 plus per cent showed a minus metabolism, the range being from minus 2 to 31 per cent with an average of minus 11 per cent.

Blood Cholesterol: Blood cholesterol estimations were made in 27 cases, the range being 171 to 300 mgms. per 100 cc. blood (Sackett's modification of Bloor's method). The average was 225 mgms.

Operations: This furnishes some interesting data. Of the 69 females, 45 or 65 plus per cent had had one or more major operations. Thirty-two or 71 plus per cent of those operated upon had had laparotomies. Of the total number operated upon, 5 or 11 per cent had had a hysterectomy for fibroid. Thirteen or close to 29 per cent had had thyroidectomies. Eight or 17.7 per cent had had cholecystectomies; of this number five had stones in the gallbladder. Four others had had a diagnosis of cholecystitis by the Graham dye method but were not operated upon. Fourteen or 31 per cent had had primary laparotomies for chronic appendicitis, two had had acute appendicitis. Eighteen or 40 per cent had had appendectomies incidental to other conditions requiring laparotomy. Three or 6 per cent had had the appendix removed and then had the gallbladder removed later, one of whom had stones. One had had three separate operations, nephropexy, appendectomy, and later cholecystectomy with stones. Three or 6 per cent had had operations for cystic mastitis. Only one had carcinoma of the

breast and she also had had a thyroidectomy previously. Two or 4.4 per cent had had cystic ovaries removed, one of whom had cholesterine clefts in the ovary and also had had a lipoma of the shoulder removed. One had had bilateral salpingectomy and another bilateral oophorectomy for unknown conditions. One had a mastoid operation and one a gastroenterostomy for duodenal ulcer. Nine or 29 per cent of the males had had major operations. Six or 66 per cent of those operated upon had appendectomies for chronic appendicitis and one for acute appendicitis. Two or 22 per cent of those operated upon had thyroidectomies. Two or 22 per cent had had herniorrhaphies, one of these having had three separate operations, viz., thyroidectomy, appendectomy, and herniorrhaphy.

Other Diseases: Twelve or 12 per cent of the one hundred cases had had multiple arthritis. Two patients, both males, showed pigment on the cornea, detached from the iris and likewise showed brownish pigmented areas on the body. Angiomas were also present. Three other patients showed definite and distinct brownish pigmented areas on the body and the face. A total of five or five per cent therefore showed pigment disturbances. Three had lipomas on the body and one of these had cholesterine clefts in the ovary. The youngest patient, a girl of nine years, had marked greying of the hair; this as well as her migraine, caused her parents to seek medical advice. Her parents both had blue irises, as also had her two brothers. Her grandfather on her father's side was 77 inches tall and her grandfather on her mother's side was 78 inches in height. One subject, 73 inches in height, had cryptorchism and his son had the same condition and presented a definite Froehlich's syndrome. One subject had keloid formation in his appendectomy scar and multiple neurofibromas such as seen in acromegaly. Only one case of diabetes mellitus was encountered. Bilateral cataracts were present in one patient. Two patients gave a history of having been jaundiced; neither of these had been operated upon, but had non-filling of the gall-bladder as indicated by the dye method. One case of hirsutism was present. Urticara and hay fever were present in three cases. Aeroparesthesia was present in two cases. Nine patients had had a diagnosis of chronic appendicitis made by fluoroscopy but had not been operated upon.

In the family history, death in the parents was most frequently caused by cardiac or vascular complications. Peculiarly enough, four of the 100 subjects stated that either the father or mother died of pernicious anemia. Three first cousins of one patient died of pernicious anemia.

Treatment: Concerning treatment, no reliable statistics can be furnished. No new or radical procedures were attempted. But it can definitely be stated that the greatest relief came from measures directed to the thyroid gland. Thyroidectomy and thyroid substance in small doses of the desiccated product such as (.013 gram) gr. one-fifth, t.i.d. gave the best results and the greatest relief. Injections of anterior and posterior pituitary lobe extracts gave no striking results.

DISCUSSION

This study confirms studies of others that inheritance is a great factor in this disease. Thirty-nine per cent gave a family history of migraine. The fact that practically all patients had an enlargement of the thyroid or definite goiter, points to some endocrine factor in this disease. The basal metabolism studies also show the thyroid element in migraine. Like in goiter there was a greater incidence (69 to 31) of women with migraine.

There are many who believe that the pituitary gland is a factor in the production of migraine. Several facts in the present study point to the pituitary as having some bearing on the symptomatology.

If we consider the heights of the immediate families in both females and males, the percentage of those who were six feet in height or close to six feet is unusually high. Seventy-five per cent of both males and females gave a family history of unusual height. If a preceding generation were included in the family history, the percentage would be close to 100. If the pituitary is in any way concerned with the skeletal development then the unusual heights are of some significance in the relationship this gland has to migraine. We have also found this height relationship in so-called idiopathic epilepsy. The blood cholesterol was somewhat elevated and here again the relationship of the pituitary to fat metabolism is to be thought of. This has been emphasized by the writer elsewhere (1).

It is rather singular that the majority of the patients insist that it is their liver or gallbladder which is the cause of the trouble, no doubt being based upon the fact that bile is vomited during the attack. Not infrequently pain in the gallbladder area is complained of.

In rats and rabbits, posterior lobe extract injections result in a very rapid increase of fat in the liver. (Coope and Chamberlain, 2.)

According to Raab (3), hypodermic injection of posterior lobe extract in dogs causes a fall in blood fat and a fatty infiltration of the liver. While, of course, this relationship between the pituitary and liver is still an unsettled point, nevertheless it provides interesting speculation in regard to the etiology of migraine.

From the data in regard to laparotomies, one may conclude that an operation for so-called chronic appendicitis is often performed and unfortunately with no relief. I was impressed with the fact that many of the patients invariably stressed the abdominal symptoms of their trouble. Twenty per cent of the hundred cases had had primary appendectomies for chronic appendicitis and in addition 9 cases had had a fluoroscopic diagnosis of chronic appendicitis but had not been operated upon. The lesson to be learned from this is that great care must be exercised in performing laparotomies on migrainous patients and this becomes particularly important in those subjects who have abortive attacks of migraine. Of further interest as pointing to a pituitary etiology is the fact that 5 per cent had definite brownish pigmented areas on the body and two of these had detached pigment from the iris on the cornea. It is, of course, well

known that the posterior lobe is in some way concerned with pigment cells.

Goiter operations are, on the other hand, justifiable in the face of toxic symptoms and fortunately many obtain relief from their headaches. Patients following thyroidectomies are able to tolerate thyroid therapy much better. It is, of course, not known how the benefit is achieved and one can only say that the thyroid influences in some way the headache, possibly affecting, among other things, the pituitary gland. This I have discussed elsewhere (1).

SUMMARY

In 100 cases of migraine, a family history of migraine was obtained in 39 per cent. Sixty-nine per cent were females, 31 per cent were males. The average age of the females was 36.8 years; of the males, 37.9 years. The duration of the disease averaged 8.35 years. The average weight of the females was 140 pounds; of the males, 160 pounds. The average height of the females was 64.6 inches, while that of the males was 70.4 inches. The immediate families of 51 females whose histories were obtained (70 per cent) approached an average of 72 inches in height. The average height of the immediate families of 21 males whose histories were obtained (80 per cent) approached 72 inches. The blood pressure averaged 129/80 in the females and 128/80 in the males. In 51 basal metabolic rate determinations in females 40 per cent gave plus readings—an average of 9 per cent. Sixty per cent of the females gave minus readings with an average of minus 10.8 per cent. In 23 basal metabolic rate determinations in males 26 per cent gave plus readings (average of 17.5 per cent), while 73 per cent gave minus readings (an average of minus 11 per cent). Twenty-seven blood cholesterol estimations gave an average of 225 mgm. Sixty-five per cent of the females had had one or more major operations. Seventy-one per cent of those operated upon had had laparotomies. Five or 11 per cent had had hysterectomy for fibroid. Eight or 17.7 per cent had had cholecystectomy. Five of this number had had cholelithiasis. Fourteen or 31 per cent had had laparotomy for chronic appendicitis. Thirteen or 29 per cent had had thyroidectomies. Nine or 29 per cent of the males had had major operations. Six or 66 per cent had had appendectomies for chronic appendicitis. Two or 22 per cent had had thyroidectomies.

CONCLUSIONS

(1) The existing evidence suggests that endocrine factors play a significant role in migraine.

(2) In one hundred cases it was noted that:

(a) The vast majority of the patients had an enlargement of the thyroid gland (but the patients lived in a goiter zone).

(b) The immediate families of the patients showed a high incidence of tallness and the male patients were above normal height (70.4 inches).

(c) The basal metabolism was frequently abnormal.

(d) The greatest relief obtained was by measures directed toward the thyroid gland (thyroidectomy and feeding of thyroid substance).

(3) Care should be exercised in performing abdominal operations on migraine patients unless the indications and findings are definite.

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THE EFFECT OF OESTRIN ON DEVELOPMENT OF THE OVARY IN IMMATURE FEMALE RATS*

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The physiological effects of the oestrous hormone on the female rat have been studied quite exhaustively and the characteristic reactions produced are generally well known. However, the literature is rather obscure on the effect of oestrin on the growth and development of the ovaries of mature and immature animals. Allen and Doisy (1) reported that the follicular hormone had the ability to induce sexual maturity in 24-day-old female rats. The effect on the ovaries was not discussed. Golding and Ramirez (2) also studied the effect of ovarian follicular hormone on immature rats. Twenty rats, including males and females from two litters, were used. Five of these were kept as controls while 15 were treated as follows: The rats were injected twice daily with 2.5 R.U. of water soluble hormone beginning on the 12th to the 18th day of life and continuing for 24 to 36 days. The dosage was increased daily by .25 R.U. The vaginas of the rats opened in 9 to 14 days. The ovaries of the oestrin treated rats appeared grossly to be smaller than those of the controls but histologically they were quite similar. They remained infantile, no ovulation having occurred, and no corpora lutea being found in the ovaries. The body weights were not affected, but the weights of the thymus glands were decreased by half in the experimental animals. These workers also state that in some females, after the injections had been discontinued, regular oestrous cycles were established.

In this paper we wish to report the effect of follicular hormone administration on the development of the ovaries of immature rats. Data are also presented to demonstrate the effect of the hormone on the weights of the thymus, pituitary, thyroid and adrenals.

PROCEDURE AND RESULTS

Sexually immature female rats from 22 to 39 days of age were injected with follicular hormone for periods of time varying from 20 to 66 days. For convenience, the period of hormone administration was used to divide the rats into groups. Immature rats were injected for 20 days and killed before they were sexually mature; others received injections from 20-41 days and were killed after they became sexually mature; still another group was injected from 41-66 days and killed after sexual maturity. In a preliminary report of this work (Leonard, Meyer and Hisaw, 3)

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this scheme of grouping was not considered. It will be shown later that the effects of the oestrous hormone on the ovaries can be demonstrated only to the best advantage when such grouping is taken into account.

The stock hormone was made up in a very concentrated form, 1 cc. equal to 500 R.U.* This was diluted with corn oil so that 0.1 cc. was equal to 2 R.U. The amount of active material was exceedingly small and probably not much inert substance, exclusive of the oil, was introduced with the hormone. For control fluid, sterile corn oil (Mazola) was used. However, in a few cases no control fluid was injected in order that the effect of the oil could be determined. The weights of the ovaries together with a histological study were the criteria used for determining the influence of the follicular hormone. In some of the litters, especially in the group that received injections beyond the time of sexual maturity, the thyroid, thymus, pituitary, and adrenals were removed and weighed.

Twelve immature female rats from 5 litters received 2 R.U. of oil soluble oestrin for a period of 21 to 40 days. The age of the rats at the beginning of the experiment ranged from 28 to 37 days. They received daily injections of oestrin beyond the time at which they became sexually mature. This has been established as occurring around 60 days in our colony of rats. Twelve litter-mate females were used as controls, six of which received 0.1 cc. of oil and six received no injections.

The vaginas of the rats which were injected with oestrin opened in 3 to 4 days after the hormone was administered, but the vaginas of the controls did not open until they were about 60 days of age. Vaginal smears of the rats injected with oestrin showed cornified or nucleated cells throughout the injection period. The rats were in good health at the end of the experiment, the average body weight being 123.7 grams for the experimental rats as compared to 127.5 grams for the controls. When the average weights of the ovaries of the oestrin treated rats from the five litters mentioned above were compared with those of their respective litter-mate controls, it was found that the ovaries of the experimental animals were 60.5, 46.6, 60.7, 38.5 and 23.3 per cent less than the controls. The average weight of the ovaries of all the experimental animals was 49.6 per cent less than the controls. (Table I.)

A second group, consisting of 8 experimental females and 9 litter-mate controls from 4 different litters, was injected with 2 R.U. of oestrin daily for a period of 41 to 66 days. The age of the rats at the beginning of the experiment ranged from 30 to 34 days and the injections were continued beyond the time of normal sexual maturity. Six of the controls received Mazola oil and three were not injected. The opening of the vagina and other manifestations of oestrum were the same as described for the preceding group. The average body weight of the treated females was 141.3 grams as compared to 152.7 grams for the controls. The corn oil produced no demonstrable change in the ovaries when compared with the non-in-

*The follicular hormone used in these experiments was amniotin, kindly furnished by E. R. Squibb & Co.

jected animals. The effect of oestrin upon the weight of the ovaries was similar to that in the first group. The ovaries of the experimental animals within the litter groups weighed 42.9, 47.0, 42.5 and 43.1 per cent less than the controls; the average for all groups was 43.0 per cent. (Table I.)

The results as described were not obtained when immature female rats were injected with oestrin for a period of 20 days and killed before they became sexually mature. Eight immature females from 4 different litters were injected from the 24th to the 44th day of life with 4 R.U. of oestrin daily. Six litter-mate controls taken from respective litters were not injected. They were all killed on the 45th day after having received 20 injections. The ovaries were removed and weighed as before. The average weight of the ovaries of the rats injected with the

TABLE I

THE EFFECT OF OESTRIN ON THE OVARIES OF SEXUALLY IMMATURE RATS WHEN THE INJECTIONS WERE CONTINUED BEYOND THE TIME OF NORMAL SEXUAL MATURITY.

Injections for 21–40 Days				Injections for 41–66 Days			
Litter	Weight of Ovary Experimental	Weight of Ovary Control	% the Experimental < Control	Litter	Weight of Ovary Experimental	Weight of Ovary Control	% the Experimental < Control
A5	7.2 11.2	33.0* 13.5*	60.5	A16	30.3 33.7	56.1*	42.9
A3	11.5 21.1	28.7 35.5 45.2* 12.8*	46.6	A18	8.0 8.0	19.3 18.5	47.0
				A23	27.1 19.2	47.7* 33.4* 45.1 43.9	42.5
A43	14.0 7.0 14.8	29.5 31.1	60.7				
				A42	45.5 19.3	49.0 65.0	43.1
A36	9.0 27.0	17.9 40.7	38.5				
						Average	43.0%
A37	11.0 11.4 20.4	15.1* 24.2*	23.3				
		Average	49.6%				

*Not injected with oil as control. Weights in mgm.

oestrous hormone was 11.3 mgm. as compared to 10.4 mgm. for the controls or 8.6 per cent heavier. The body weights remained practically the same, averaging 95.4 grams for the experimental rats and 96.6 grams for the controls. The vaginas of the control rats had not opened on the 45th day of life and macroscopic examination showed no corpora lutea in the ovaries of either the experimental or control animals (Table II).

This experiment was repeated, using corn oil for control injections. Nine immature females, 22 to 25 days of age, were selected from 4 litters, and given a daily dose of 2 R.U. of oestrin in 0.1 cc. of Mazola oil. The same amount of oil was injected into 6 litter-mate sisters. The injections were continued for 20 days, the animals killed, and the ovaries and thymus glands were weighed. The average weight of the ovaries of the rats that

received oestrin was 10.8 mgm., which is 23.4 per cent lighter than those of the controls weighing 14.1 mgm. The body weights remained practically unaffected, averaging 82.9 grams for the experimental animals and 86.3 grams for the controls. The average weight of the thymus glands in the experimental females of this group was 0.2496 grams as compared to 0.2686 grams for the controls. This is a difference of only 7 per cent, which may not be significant.

Some of the other endocrine glands from those groups of animals which received injections beyond the time of normal sexual maturity were also weighed for comparison. These included the thyroid, thymus, pituitary, and adrenals (Table III). The results for 8 experimental rats and 9 litter-mate controls showed that the weight of the glands was, perhaps, not affected significantly by the injection of oestrin. In average percent-

TABLE II

THE EFFECTS OF OESTRIN ON THE OVARIES OF SEXUALLY IMMATURE RATS WHICH WERE TREATED FOR 20 DAYS AND KILLED BEFORE SEXUAL MATURITY.

Without Oil Control				With Oil Control			
Litter	Weight of Ovaries Experimental	Weight of Ovaries Control	% the Experimental < Control	Litter	Weight of Ovaries Experimental	Weight of Ovaries Control	% the Experimental < Control
A67	9.2	10.9	15.6	A132	9.9 8.5 7.2	10.9 12.4	26.8
A68	11.7 10.0	12.0	9.6	A137	10.1 13.7	15.9	25.2
A71	15.0 13.3	11.0	+28.6	A134	11.1 12.6	15.1	21.6
A70	9.7 12.1 9.7	8.6 12.3 7.7	+ 9.5	A138	13.7 10.5	15.5 14.9	20.4
Average...	11.3	10.4	+ 8.6%	Average..	10.8	14.1	23.4%

Weights in mgm.

ages, the thyroids were 5.2 per cent lighter, the thymus 13.5 per cent lighter, the adrenals 0.6 per cent heavier and the pituitary 14.5 per cent heavier than the controls. These differences appear to be within the limits of normal variation and do not indicate at least the marked influence of oestrin which is so striking in the case of the ovaries.

On examining the histological sections of the ovaries from all groups no great changes were noted. The ovaries of the animals which received oestrin and were killed before the attainment of sexual maturity, appeared normal when compared with those of their litter-mate controls. Young ovarian follicles, atretic follicles, and the absence of luteal tissue was the usual condition. However, the ovaries from the experimental rats in the groups that were killed after attaining sexual maturity showed a marked diminution in the number of ripe follicles and formed corpora lutea. Evi-

TABLE III

Effect of Oestrin on Weight of other Endocrine Glands.

Litter	Rat	No. of Projections	Experimental					Control				
			Weight of Ovaries	Weight of Pituitary	Weight of Thyroid	Weight of Thymus	Weight of Adrenals	Weight of Ovaries	Weight of Pituitary	Weight of Thyroid	Weight of Thymus	Weight of Adrenals
A10	L_N	37	9.1	3.9	15.8	224.5	29.0					
	Lo	37	11.2	4.0	12.9	185.7	28.4					
	R_N	37						21.7	4.1	19.1	275.4	30.1
	R_{2N}	37						40.7	5.2		246.9	29.6
	Ro	37						43.5	6.1	23.0	216.3	36.9
A11	L_N	40	26.4	6.5	12.0	268.5	35.9					
	L_NR_N	40	12.9	7.1	14.2	404.5	32.0					
	R_N	40						14.3		18.7	331.7	31.5
A5	R_N	40	7.2	7.3	9.7	237.6	45.8					
	Lo	40	11.2	6.0	13.5	192.5	32.5					
	L_N	40						33.0	2.0	10.5	272.5	40.2
	Ro	40						13.5	4.8	7.5	236.9	27.2
A3	R_N	33	11.5	7.6	11.1	281.5	31.5					
	R^3_N	33	21.1	7.9	11.2	268.1	50.6					
	L_{2N}	33						28.7	7.9	8.3	355.8	29.6
	L_N	33						35.5	6.5	3.5	335.5	33.4
	R_{2N}	33						45.2	7.8	16.3	413.7	43.2
Average			13.8	6.3	12.6	257.9	35.7	30.7	5.5	13.3	298.3	35.5
Per Cent Difference			—55.0%	+14.5%	— 5.2%	—13.5%	+ 0.6%					

All weights in mgm.

dently there was a decrease in the number of ova to reach maturity and to be ovulated, with the subsequent formation of corpora lutea. Other than this the ovaries appeared the same as in the controls.

DISCUSSION

It is apparent from this study that administration of follicular hormone, when begun in early life and continued beyond the time of normal sexual maturity, prevents full development of the ovaries. The ovaries of the experimental animals weigh, on the average, 43 to 49 per cent less than the normals. This decrease in weight is the result of a diminished number of follicles reaching maturity and a subsequent smaller number of corpora lutea. This is in reality a partial inhibition of ovulation and of follicular maturation. Haberlandt (4) claims to have produced sterility in white mice by the administration of placental hormones by mouth. Dahlberg and Akesson (5) have, in two cases, fed follicular hormone to an immature mouse and prevented it from attaining sexual maturity at the proper age. Thus it seems that the follicular hormone can prevent ovulation partially or wholly, according to the amount given.

When follicular hormone is given to immature rats which are killed before the time of normal sexual maturity, there are no outstanding modifications of the ovaries. In one series the experimental animals had ovaries 8.6 per cent heavier, a result which is within the range of normal variation, and in the other they were 23.7 per cent lighter than their respective controls. The 23.7 per cent difference in ovarian weights seems to have been due to the beginning of follicular development in the controls and its inhibition in the experimental animals. However, this difference does not approach that found for animals which were sacrificed after they became sexually mature.

The weights of the other endocrine glands which were considered in this study were apparently unaffected by the follicular hormone. Thus it seems that the ovary is the only gland whose weight is significantly influenced. For example, the thymus was found to be decreased by 7 per cent in one series and 13.5 per cent in another. In neither case does this compare with the work of Golding and Ramirez (2), who report a 50 per cent decrease in the weight of the thymus as a result of oestrin treatment in an experiment similar to the one reported here. Whether oestrin has a specific influence on the thymus gland may be questioned. It has been found in this laboratory that several substances, particularly if toxic, cause a decrease in weight of the thymus and that some of these substances are much more effective than oestrin. Possibly the follicular hormone used in previous work contained some toxic material.

One may question the use of weight as a dependable physiological criterion. It seems reasonable to think that it may be employed with accuracy where gross anatomical differences are concerned, but certainly not as a measure of physiological activity of the parts affected. This is

quite well illustrated here. The average weight of the thyroid and adrenals remained practically unchanged, while the average weight of the pituitary was 14.5 per cent heavier in the groups studied. It might be concluded from these data that the pituitary would have an increased physiological activity as determined by the implant method (6). However, such an assumption was disproven. The pituitaries of the rats receiving the oestrous hormone were much less efficient in producing sexual precocity when transplanted into sexually immature females than those of their litter-mate controls (7). This decrease in the gonad stimulating hormones of the pituitary can also be detected in the group which received oestrin and was killed before sexual maturity. It is interesting to note that oestrin can affect the gonad stimulating power of the pituitary before the latter has initiated oestrous cycles in the immature rat.

It is concluded from the data obtained in this study that the ovary is more sensitive to oestrin than the other endocrine organs examined. Whether this is a direct or an indirect effect is a question of importance. The smaller weight of the experimental ovaries is a result of decrease in follicular development and in the number of corpora lutea. These facts are correlated with a decrease in gonad stimulating power of the hypophysis. The fact that the follicular hormone can influence the anterior pituitary in decreasing its gonad stimulating power suggests an antagonism between the follicular hormone and the hormonal complex of the anterior lobe. Such a relationship might be well incorporated in a theoretical explanation of the mechanism of the rhythmical action of the ovary found in the normal cycle wherein there is a rise and fall in the production of the follicular hormone. Then the effects of oestrin on the ovary may not be direct, but by way of the hypophysis. If this is upheld by subsequent work, it will contribute to the present rather vague ideas of endocrine interrelationships.

CONCLUSION

The injection of follicular hormone prevents the ovaries of immature rats from reaching full growth and development as indicated by the weight of the glands. This effect cannot be readily detected unless the injections are continued beyond the time the animals become sexually mature. The weights of the pituitary, thymus, thyroid and adrenals remain practically unchanged. Physiologically, the pituitaries of rats receiving oestrin have been found to be less potent in their ability to induce sexual precocity in immature female rats, and thus, by its influence on the hypophysis, oestrin inhibits the development of the ovary.

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SUPPRESSION OF GLUCOSE COMBUSTION BY EPINEPHRIN ADMINISTRATION*†

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In most respects epinephrin appears to interfere with normal utilization of carbohydrate in the body. It causes hyperglycemia and glycosuria and relieves insulin hypoglycemia, effects which may be accounted for by increased mobilization of glycogen or inhibition of glycogen storage or both. It might be anticipated, therefore, that it would also have a depressing influence on combustion of glucose inasmuch as storage and combustion usually, i. e., in diabetes and after insulin administration, go hand in hand. Yet that idea fails to find support in the literature. After epinephrin in single doses subcutaneously the total heat production increases and the carbon-dioxide elimination rises, as a rule, promptly and transiently (1). It has not been determined whether the succeeding increased respiratory quotient results from increased glucose combustion or from washing out of carbon-dioxide due to increased lactic acid production (2) or over-ventilation (3) or both. It is perhaps significant that the plasma bicarbonate concentration falls simultaneously (4).

With one exception (5), existing reports give no hint of interference with glucose combustion by epinephrin. In view of the experiments to be reported herewith this appears to be due to the fact that the time factor has not been taken into account previously. When it is considered that after pancreatectomy, for example, or after insulin administration in diabetes, major variations in glucose oxidation are not manifest for several hours, it seems not unreasonable to expect that epinephrin in prolonged dosage might prove to have effects not shown by single doses. This possibility has been investigated with results which indicate that continuous epinephrin administration may actually suppress glucose oxidation completely.

METHODS

All observations were made on female cats uniformly anesthetized by means of amytal. Constant anesthesia could be maintained for many hours by means of an initial anesthetic dose intraperitoneally followed by a sustained intravenous supply of about 3 mgm. per kgm. per hour. Within rather wide limits the depth of anesthesia did not appear to affect the constancy of the results. Easily recognized extremes of anesthesia such as increased responsiveness and shivering on one hand or respiratory and circulatory depression on the other were avoided as a rule. Their occur-

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rence was considered cause for rejection of findings (6). Experience gained with this method in about 130 experiments lasting from 6 to 48 hours made it possible to eliminate errors from this source.

The experimental method has been described in detail previously (6, 7). Briefly, the constant intravenous injection method introduced by Woodyatt, Sansum and Wilder (8) was used in various ways. The response to glucose alone and to glucose with added epinephrin was determined in consecutive half-hourly or hourly periods. With a constant glucose supply the excretion was measured directly and the oxidation was computed from the non-protein respiratory data (7).*

RESULTS

Figure 1 is typical of a dozen or more experiments with glucose alone. After a preliminary hour or two the sum of the glucose oxidized and excreted was constant, ordinarily accounting for 70 to 90 per cent of the

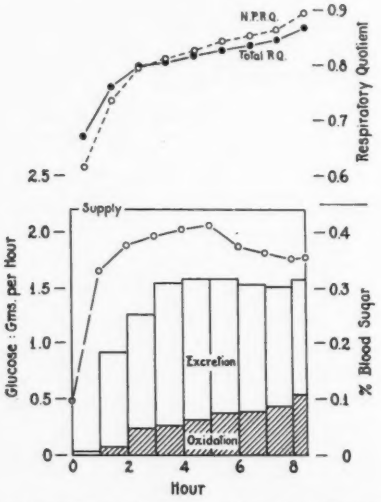

Fig. 1. Oxidation and excretion of glucose when the intravenous supply is constant. Cat 319, weight 2.40 kgm. Diet, milk and lactose only for 3 days previously. Glucose supply: 21.8 cc. 10.1 per cent solution or 2202 mgm. per hour = 0.92 gram per kgm. per hour.

supply. The balance was presumably stored as glycogen at a constant measurable rate since the blood sugar concentration was also maintained at a fairly constant level after the initial rise. Beginning at a low level, the quantity of glucose oxidized per hour invariably rose slowly but progressively in a manner qualitatively identical with that in unanesthetized preparations. In some cases, as in the one illustrated, the respiratory

*The validity of the assumption that the non-protein respiratory exchange depicts accurately the rate of glucose oxidation under these conditions is admittedly open to question. It should be noted that the values for oxidation as well as the conclusions which are based upon them involve this assumption, and are therefore cogent only insofar as respiratory data are acknowledged to yield information concerning glucose combustion.

quotient rose only to a moderate degree while in others it exceeded unity, indicating fat formation (8). Without epinephrin it never failed to show a typical response to glucose administration.

The effect of a single intravenous dose of insulin during glucose administration is shown in figure 2. After 6 hours of glucose the oxidation and excretion were fairly constant and their sum represented the usual major portion of the supply. Two units of insulin in a single dose by vein resulted in a transient increase in oxidation and a more prolonged diminution in excretion, and polymerization into glycogen, taken as the glucose unaccounted for, proceeded at an increased rate, outlasting the increased combustion.

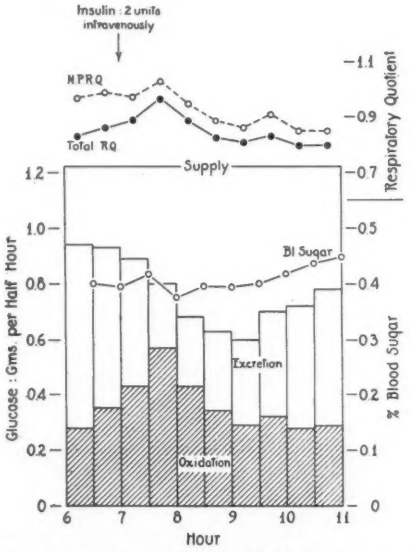

Fig. 2. Effect of insulin on the utilization of injected glucose. Cat 313, weight 3.00 kgm. Glucose supply: 24.1 cc. 10.1 per cent solution or 2.43 grams per hour = 0.81 gram per kgm. per hour. Insulin: 2 units intravenously at end of sixth hour of injection.

Transient epinephrin administration produced the results illustrated by figure 3. After 5½ hours of glucose alone the customary constancy of oxidation, excretion and polymerization was established. Epinephrin, given intravenously at a rate of 0.001 mgm. per kgm. per minute for 15 minutes, resulted in temporary increases in the excretion and combustion rates, and, inasmuch as their sum exceeded the supply and the blood sugar concentration rose, the rate of mobilization of glucose from glycogen may be considered to have increased. This response is quite similar to the effects, reported by others, of a single dose of the drug given subcutaneously.

In the next three experiments the conditions were identical with those of the preceding except that epinephrin was added to the glucose in quan-

tities which would result in the constant intravenous administration of 0.001 mgm. per kgm. per minute from the beginning of the glucose injection over a period of hours. The dosage selected is well within the maximum physiological range. Cannon and Rapport (9) and Kodama (10) separately observed epinephrin secretion rates as great as 0.005 mgm. per kgm. per minute in cats after various types of afferent stimulation, or five times the dosage employed. No gross circulatory effects were noted in any case.

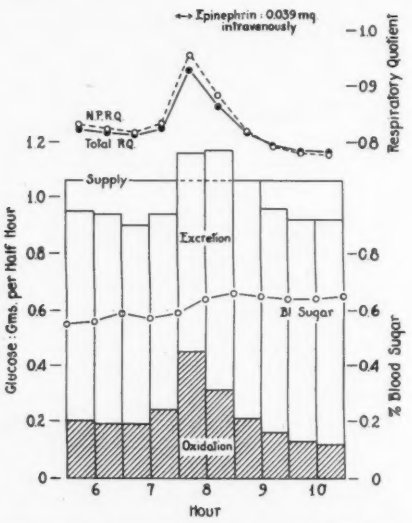

Fig. 3. Effect of epinephrin in transient dosage. Cat 312, weight 2.60 kgm. Glucose supply: 21.0 cc. 10.1 per cent solution or 2.12 grams per hour = 0.82 gram per kgm. per hour. Epinephrin: 0.039 mgm. intravenously during third quarter of eighth hour of injection.

Figures 4, 5 and 6 illustrate the altered response due to continuous epinephrin administration. In spite of the continuous supply of glucose, instead of rising progressively as with glucose alone, the rates of oxidation invariably fell off, with minor variations, until, according to the respiratory data, glucose combustion was virtually abolished, the total and nonprotein respiratory quotients resting near the fat level. The excretion rates were high, even though in two instances the rates of glucose injection would ordinarily (without epinephrin) cause no abnormal glycosuria whatsoever. A variable quantity of extra glucose could be accounted for initially, indicating glycogenolysis, and later the excretion tended to become constant at the supply level. This total excretion of the supply further suggests that no glucose was being utilized, confirming the respiratory data.

In the experiment illustrated in figure 6, after the usual effects of epinephrin had been established its administration was stopped and the glucose continued at the same rate. This resulted in a prompt accelera-

tion of glucose combustion, verifying the supposition that the previous suppression of oxidation was in fact caused by the epinephrin. It is of interest to note an analogy here. Withdrawing the epinephrin produced effects identical with those produced by a dose of insulin in a case of total diabetes. Oxidation of sugar was augmented, glycosuria, hyperglycemia and nitrogen excretion were decreased, glycogen (taken as the missing sugar) was stored, and the time relations of these events were comparable to those of the action of insulin in diabetes.

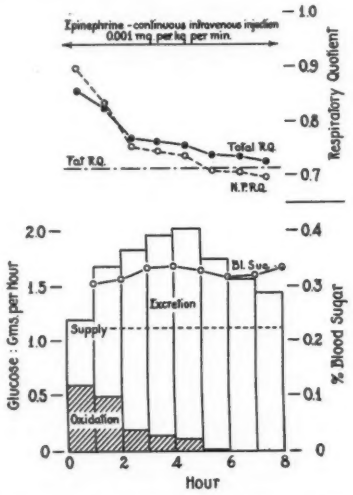

Fig. 4. Suppression of glucose oxidation by continuous administration of epinephrin, in spite of excessive glucose supply. Cat 325, weight 2.64 kgm. Diet, exclusively milk and lactose for 3 days previously. Glucose supply: 10.8 cc. 10.3 per cent solution or 1110 mgm. per hour = 0.42 gram per kgm. per hour. Epinephrin supply: 0.16 mgm. per hour = 0.001 mgm. per kgm. per minute.

It could scarcely be maintained that the significance of these findings is entirely clear. Naturally one of the first ideas to suggest itself is that of inhibition of insulin secretion by epinephrin, a virtual suppression of internal pancreas secretion by a hormonal mechanism. According to this theory continuous epinephrin administration should duplicate the results of removal of the pancreas as far as *carbohydrate metabolism* is concerned, i. e., when given to a fasting animal it should cause continuous glycosuria, originally from glycogen and ultimately from protein, with constant and proportional excretion rates of glucose and nitrogen.

Although some technical difficulties were encountered, several attempts to test this idea were made under the experimental conditions employed. Anesthetized animals were given epinephrin in isotonic saline intravenously at constant rates in dosages of 0.001 to 0.002 mgm. per kgm. per minute for variable periods up to 48 hours and the resulting glucose and nitrogen excretion rates observed. The animals had been fasted previously to deplete their glycogen reserves and no glucose was injected with

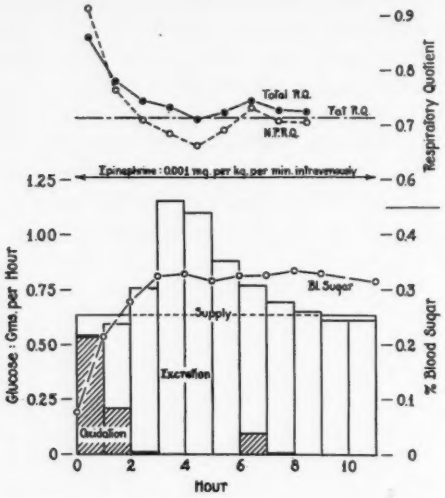

Fig. 5. Suppression of glucose oxidation by continuous epinephrin supply, in spite of continuous glucose supply. Cat 329, weight 2.45 kgm. Deprived of food 72 hours previously. Glucose supply: 12 :6 cc. 5.00 percent solution or 628 mgm. per hour = 0.26 gram per kgm. per hour. Epinephrin supply: 0.15 mgm. per hour or 0.001 mgm. per kgm. per minute.

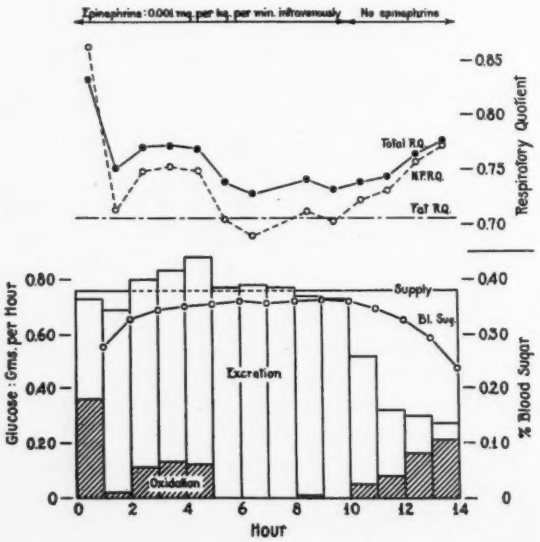

Fig. 6. Suppression of glucose oxidation by continuous epinephrin administration, in spite of continuous glucose supply. Subsequent recovery of oxidation upon withdrawing epinephrin. Cat 326, weight 2.32 kgm. Diet, milk and lactose only for 2 days previously. Glucose supply: 15.1 cc. 5.00 per cent solution or 760 mgm. per hour = 0.33 gram per kgm. per hour. Epinephrin supply: 0.15 mgm. per hour = 0.001 mgm. per kgm. per minute for first 10 hours only.

the epinephrin. Gross glycosuria invariably occurred promptly, reached a maximum in a few hours and in all instances but one disappeared after 15 to 23 hours. The blood sugar concentration followed an approximately parallel course but tended to remain slightly elevated (0.16 to 0.20 per cent) after abnormal glycosuria had ceased. Nitrogen excretion persisted unchanged in all cases, even after cessation of glycosuria.

One suggestive set of results was obtained, however (figure 7). In this experiment after the 13th hour of epinephrin alone the glycosuria and hyperglycemia became constant for six successive hourly periods. During this time the nitrogen excretion also was constant and the D to N ratio was consequently maintained uniformly at a level of 1.1. The fact

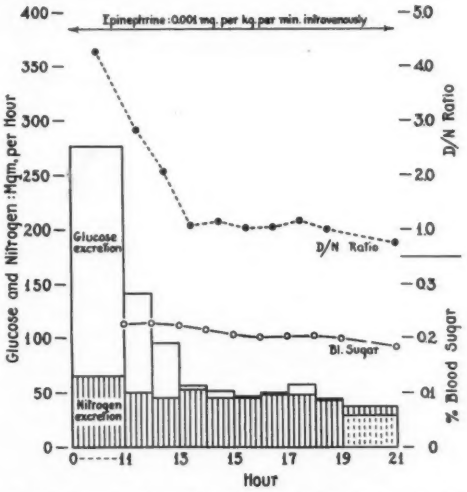

Fig. 7. Effect of continuous epinephrin administration during fasting. Cat 332, weight 2.30 kgm. Deprived of food 72 hours previously. Epinephrin administration: 6.9 cc. per hour of solution containing 2.0 mgm. epinephrin per 100 cc. = 0.138 mgm. per hour or 0.001 mgm. per kgm. per minute, given intravenously. Signs of circulatory failure after 19th hour. Cat died 6 hours after end of experiment. Values for glucose and nitrogen are superimposed.

that all values fell off perceptibly after the 19th hour can not be considered seriously since the animal began simultaneously to show signs of circulatory failure and died 6 hours later.

Several attempts to recover single doses of glucose injected at the height of the epinephrin influence, i. e., after the initial glycosuria had subsided, were likewise indecisive. In one case about 400 of the 500 mgm. injected appeared in the urine within 4 hours. Four other attempts resulted in recoveries of 25 per cent or less.

A word might well be said in regard to the anesthesia used in these experiments. Probably it will be asserted that results obtained under artificial conditions such as these are not necessarily applicable to normal physiology. To this objection there is little to be said in defense of the present results. They should be confirmed under other conditions if pos-

sible. The technical difficulties involved in a satisfactory test of the question appear to preclude the possibility of using unanesthetized preparations. Even if that were possible, it seems doubtful whether the complicating effects of anesthesia are any less desirable than the emotional difficulties which would be encountered in similar experiments on normal animals. The fact remains that *in spite of the conditions employed glucose oxidation does not fail to proceed normally,* whereas under identical conditions *with a continuous supply of epinephrin in physiological dosage,* judged by the usual criteria, *it is suppressed completely.*

Further crucial experiments are necessary before any decisive explanation can be applied to these results. Other interpretations than that of insulin suppression might be offered, for example, a disturbance of the peripheral vascular bed or a specific stimulating effect on fat metabolism. The evidence presented by Cori and Cori (11) which indicates that epinephrin probably increases glycogen mobilization and inhibits glycogen synthesis in the muscles might appear to explain the phenomenon of suppressed oxidation on the basis of glycogen depletion alone. The depletion is never complete, however. Consequently if no other mechanism were involved some oxidation of muscle glycogen might be expected to persist. Furthermore, as pointed out by the Coris (12), their results with epinephrin might also be accounted for on the basis of abolition of insulin production.

The insulin hypothesis merits further consideration because of the fact that a number of other isolated clinical and experimental observations are capable of explanation by it. Some of these have been discussed elsewhere (13). It is sufficient to state here that if it should prove to withstand critical examination it might be of significance in connection with the etiology of diabetes mellitus.

SUMMARY

Cats under uniform anesthesia with amytal were used exclusively. Glucose, administered intravenously at constant rates of from 0.8 to 1.0 mgm. per kgm. per hour over periods of eight hours or longer, never failed to cause a rise in the respiratory quotient and hence in the calculated rate of glucose combustion. Ordinarily from 70 to 90 per cent of the administered glucose could be accounted for, after a preliminary period of at most three hours, by oxidation and excretion (and in a few cases also by conversion into fat).

Under otherwise identical conditions upon addition of epinephrine to the injected solutions, the respiratory quotient, and hence the estimated rate of glucose combustion, never failed to fall. After three to five hours of glucose and epinephrine the non-protein quotient assumed a level near 0.7 and tended to remain fairly constant as long as the epinephrine was continued. During that interval the excretion of glucose also tended to equal the supply. Upon stopping the epinephrin (and continuing the glucose) the quotient rose progressively for at least four hours. In one

experiment the continued administration of epinephrin without glucose led to glycosuria which reached its maximum during the first eleven hours, then fell until the thirteenth hour, after which it became constant for six hours. During the latter period the glucose and nitrogen excretions were proportional, in the ratio of 1.1 to 1.

The epinephrin dosage employed was 0.001 mgm. per kgm. per minute, or about one-fifth the maximum physiological secretion rate during reflex stimulation of the adrenals.

The experiments are interpreted as indicating that continuous epinephrin administration in physiological quantities interferes with, and may even abolish, glucose combustion. Thus it tends to suppress total glucose utilization, possibly by suppressing the secretion of insulin.

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THE EFFECTS OF ABDOMINAL SYMPATHECTOMY, ADRENAL INACTIVATION AND REMOVAL OF THE STELLATE GANGLIA ON THE SPONTANEOUS ACTIVITY OF THE ALBINO RAT

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It is well known that certain endocrine secretions play an important rôle in the regulation of the level of spontaneous activity of the ablino rat. Removal of the adrenals (Durrant, 1), testes (Hoskins, 2), or ovaries (Wang, 3) results in a marked decrease in activity. Thyroidectomy, however (Lee and Van Buskirk, 4), apparently does not affect the activity level. Since these endocrine glands are innervated by the sympathetic system, it was thought important to investigate the question of the effect of sympathectomy on the spontaneous activity. Recently, Richter and Wislocki (5) found that rats with total removal of the pituitary gland are very inactive, but the innervation of the hypophysis is still largely unknown. Furthermore, in some of our rats, the sympathetic nerve supply to the four limbs was also destroyed, and the data here reported may have some importance in relation to the much debated question of the sympathetic innervation of the skeletal muscles.

METHODS

Revolving cages with automatic counters, of the type described by Durrant (1) were used to measure the spontaneous activity of the animals. An adequate diet was given and the room temperature was maintained around 75 degrees F. Previous to the operation the rats were kept in the cages for a preliminary period of 17 to 30 days in order to determine their normal activity levels. On a certain number of control animals, sham operations were performed. These served to forestall being misled by unnoticed changes in environmental conditions and also to give a comparison of the influence of sympathectomy with that of the traumatism.

The technique of the various sympathectomy operations in the rat will be reported later in connection with the study of the effect of this operation on reproduction and lactation. The abdominal chains were removed from the pelvic ganglia to the second lumbar ganglia, inclusively. The right adrenal was removed and the left was inactivated by severance of the left splanchnic nerve and removal of the left coeliac ganglion. Both stellates were removed and, usually, the upper thoracic chains down to the level of the third intercostal space. All of the operations were performed

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under ether anesthesia. Postmortem examinations revealed in every case regeneration of the preganglionic fibres going to the left adrenal. No regeneration of the thoracic chain could be detected. Whether or not there occurred a regeneration of the preganglionic fibres extending from the abdominal chains to the inferior mesenteric ganglion could not be determined, the presence of cicatricial tissue making it very difficult to detect small sympathetic fibres.

Spontaneous activity after abdominal sympathectomy. The animals used in these experiments were about 16 months old at the time of the operation. Their activity had reached a constant low plateau. Some very sluggish males were sympathectomized in order to see if the enlargement of the seminal vesicles which occurs after abdominal sympathectomy (Bacq, 6) had any stimulating effect on their activity. The results on the sympathectomized rats and five controls are reported in Tables 1 and 2. During the first days following the operation, the activity of the sympathectomized animals dropped, but a similar decrease was observed in the controls. Subsequent periods showed slightly increased activity in both sympathectomized and control rats. No definite increase in number of revolutions was recorded in the old sluggish males.

TABLE I

SPONTANEOUS ACTIVITY OF ALBINO RATS AFTER ABDOMINAL SYMPATHECTOMY.

Rat Number	Average Daily Activity 10 Days Before Operation	Average Daily Activity After Operation By Periods of 10 Days				
	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions
403 F.	1250	237	352	343	445	445
73 F.	1688	2530	4659	3147	3004	3321
76 F.	3818	4421	5570	6254	5397	5057
413 F.	5332	2574	3849	4245	4100	4560
421 M.	517	304	375	427	493	470
486 M.	80	64	119	114	130	130
425 M.	131	689	540	823	314	369
456 M.	39	49	58	44	14	18
440 M.	748	198	323	112	100	168
441 M.	129	74	97	165	183	185
Average.....	1383	1114	1594	1567	1418	1472
Per Cent Change from Preliminary Period.....		−20	+15	+13	+ 3	+ 7

TABLE II

SPONTANEOUS ACTIVITY OF ALBINO RATS AFTER CONTROL OPERATION IN THE ABDOMEN.

Rat	Average Daily Activity 10 Days Before Operation	Average Daily Activity After Control Operation By Periods of 10 Day s				
	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions
423 F.	5297	5778	6336	7957	6494	5988
409 F.	4503	2975	4868	4881	4442	5841
74 F.	511	433	545	386	398	432
448 M.	30	39	49	49	48	24
443 M.	161	82	77	57	54	64
Average.....	2100	1861	2375	2666	2287	2470
Per Cent Change From Preliminary Period.....		−12	+13	+27	+ 9	+18

The result of the abdominal sympathectomy, as performed in the rat, is chiefly to deprive the genital organs and the hind limbs of their sympathetic innervation. The adrenals are not inactivated.

Spontaneous activity after abdominal sympathectomy and adrenal inactivation. Table 3 shows the data on spontaneous activity in six fe-

TABLE III

SPONTANEOUS ACTIVITY OF ALBINO RATS AFTER ABDOMINAL SYMPATHECTOMY AND ADRENAL INACTIVATION. (FIRST SERIES)

Rat	Average Daily Activity 10 Days Before Operation	Average Daily Activity After Operation By Periods of 10 Days				
	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions
434 F.	6315	1670	2800	2810	3560	3560
406 F.	1826	1167	3046	3919	3823	4609
401 F.	3612	1620	4770	4813	3617	3698
7 F.	5460	1684	6174	6660	7555	10495
6 F.	5763	3977	5302	5528	4973	5779
5 F.	4817	2912	5595	5558	5006	4848
Average.....	4632	2005	4615	4881	4756	5448
Per Cent Change From Preliminary Period.....		—57	=	+ 6	+ 3	+18

males, before and after abdominal sympathectomy and adrenal inactivation. These animals were about 15 months old at the time of the operation. Only during the first 10 days following the operation was a lowered activity observed. During the next 40 days the average daily activity was not significantly different from that shown during the 10 days before the operation. Unfortunately, the death of two control rats resulted in the impossibility of having a sufficient number of controls, and we decided to sympathectomize a new series of animals. The results are given in Tables 4 and 5. The experiment began during the period of high activity which is characteristic of young animals. After the operation, the activity of both sympathectomized and control rats dropped markedly. As com-

TABLE IV

SPONTANEOUS ACTIVITY OF ALBINO RATS AFTER ABDOMINAL SYMPATHECTOMY AND ADRENAL INACTIVATION. (SECOND SERIES)

Rat Number	Age at the End of Preliminary Period	Average Daily Activity 30 Days Before Operation	Average Daily Activity After Operation By Periods of 10 Days				
	Days	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions
46 M.	240	5296	2087	1878	1522	761	336
72 M.	180	16413	5064	5607	5112	4538	2049
83 M.	175	5937	1976	2187	1643	1768	1305
35 F.	231	7535	3593	5429	4191	3403	3649
63 F.	271	11005	1339	3162	2722	4649	6130
65 F.	181	16889	2612	8540	7059	7612	5247
71 F.	181	7913	2176	3123	2233	2081	963
Average...	208	10141	2692	4333	3497	3476	2811
Per Cent Change From Preliminary Period.....			—73	—57	—66	—66	—72
Per Cent Difference From Controls			—16	— 6	— 6	+15	+15

TABLE V

SPONTANEOUS ACTIVITY OF ALBINO RATS AFTER CONTROL OPERATION IN THE ABDOMEN. (SECOND SERIES)

Rat Number	Age at the End of Preliminary Period	Average Daily Activity 30 Days Before Control Operation	Average Daily Activity After Control Operation By Periods of 10 Days				
	Days	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions
47 M.	240	4643	4635	2729	4072	1450	164
52 M.	229	7541	2695	2924	1967	1220	783
50 F.	241	7714	2085	3638	3253	1751	1450
70 F.	185	5411	1564	3174	781	509	838
Average...	224	6327	2745	3116	2518	1233	809
Per Cent Change From Preliminary Period.....			—57	—51	—60	—81	—87

pared with the controls, the activity of the sympathectomized animals was noticeably lower during the first 10 days after the operation, about the same during two subsequent periods of 10 days, and higher during the two following 10-day periods. It is probable, however, that this higher activity recorded in the sympathectomized animals is only apparent, and due to the fact that two of the control animals were at that time in rather poor condition. From the data given in Tables 3 and 4 it would appear that the action of abdominal sympathectomy and adrenal inactivation is to reduce the spontaneous activity slightly more than a sham operation, during a short period of 10 days.

Spontaneous activity after removal of one or both stellate ganglia. Removal of the stellates, usually with the upper part of the thoracic chain, down to the third intercostal space, was performed on rats whose abdominal sympathetic chains had previously been removed. Hence, Tables 6 and 7 give further data on the same animals as those in Tables 1, 2 and 3 (one normal rat only was added to the controls). This operation is quite severe. After artificial respiration continued for about 45 minutes and opening of the chest, rats usually show gasping during a few days. It was to be expected that the activity of both controls and sympathectomized animals should drop. But again the decrease in activity of the two series was about parallel. Only during the first 10 days following the operation was the decrease in activity more marked in the sympathectomized rats.

DISCUSSION

Obviously by the removal of the sympathetic nerve supply to the genital organs and by adrenal inactivation, we failed in reproducing the drop in activity observed in rats after castration, spaying or double adrenalectomy. The oestrus cycles with their peaks of activity persist after abdominal sympathectomy and adrenal inactivation, as shown in Figure 1 (confirming Sweet and Thorp's observations with the vaginal smear method, 7). Furthermore, as will be reported in another paper, fecundation, gestation, parturition and, in most cases, lactation are not affected by abdominal sympathectomy (severance of preganglionic fibres),

TABLE VI

Spontaneous Activity of Albino Rats After Removal of One or Both Stellates

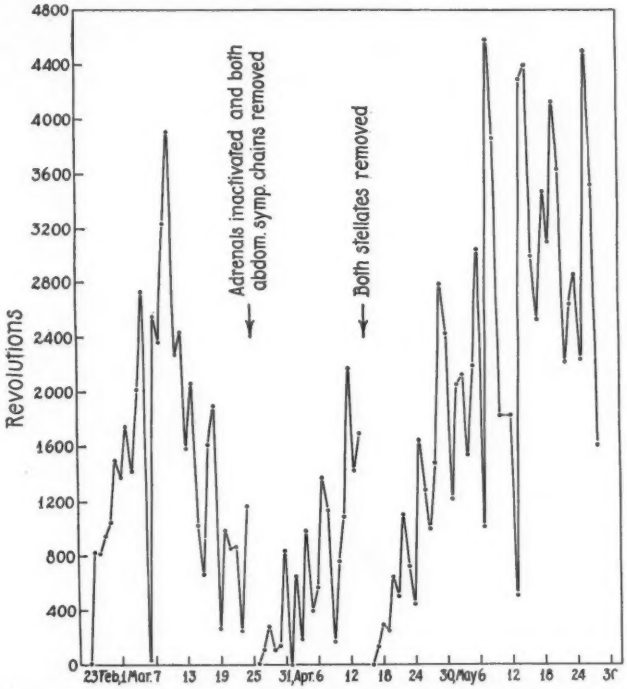
Rat Number	Stellates Removed	Average Daily Activity 10 Days Before Operation	Average Daily Activity After Operation By Periods of 10 Days							
		Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions
403 F.	Right	445	139	65	81	70	134	99	79	....
73 F.	Right	3321	1714	2611	5960	6017	6322	4148	5668	6342
76 F.	Both	5057	772	4418	2047	5495	3943	4864	3799	2960
413 F.	Both	4560	900	1353	4571	5581	4011	3419	4408	2689
421 M.	Both	470	153	449	532	276	90	35	38	34
486 M.	Right	130	151	171	117	134	49	47	23	53
434 F.	Both	3560	321	1248	2066	2766	3566	6469	6325	7624
406 F.	Both	4609	1540	2339	3008	6399	4108	4620	5039	5529
401 F.	Both	3698	1036	1230	987	894	698	1369	1668	962
7 F.	Both	10495	3141	7105	6459	6519	6482	7145	8210	8908
6 F.	Both	5779	1257	4497	4718	4348	3797	4568	4043	5041
Average		3829	1011	2408	2777	3500	3017	3573	3573	3649
Per Cent Change From Preliminary Period			—74	—37	—27	— 9	—21	— 7	— 7	— 5
Per Cent Difference From Controls			—26	— 8	+ 2	+ 9	— 8	— 2	....	....

TABLE VII

Spontaneous Activity of Albino Rats After Sham Operation in the Chest.

Rat Number	Operated Sides	Average Daily Activity 10 Days Before Operation	Average Daily Activity After Operation By Periods of 10 Days							
		Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions	Revolutions
74 F.	Right	423	89	301	301	300	473	297	273	316
423 F.	Right	5988	4152	5104	6595	6852	7412	8175	7698	7226
409 F.	Both	5841	3906	3375	1290	3818	5313	6050	4824	4053
402 F.	Both	14213	5508	10053	10649	10815	10977	10671	....	....
Average		6626	3414	4708	4709	5446	5794	6298	....	....
Per Cent Change From Preliminary Period			—48	—29	—29	—18	—13	— 5	....	....

and further observations have shown that removal of the inferior mesenteric ganglion and the whole mesenteric plexus (severance of postganglionic fibers) also apparently leaves intact the various functions of the ovary. The activity of the males is unchanged after sympathetic denervation of the genital organs. We have shown elsewhere (6) that males subjected to this operation are impotent because of their inability to ejaculate seminal fluid. No degeneration of the testes was detectable by histological examination. Thus no evidence of any sympathetic control of the internal secretion of the testes has been collected.



Spontaneous activity of Wistar rat 550, 156 days old at the beginning of the experiment.

That adrenal inactivation does not induce a lack of vigor indicates again that it is probably the cortex and not the medulla which influences the spontaneous activity of the rat. Indeed, the adrenal inactivation combined with abdominal sympathectomy and removal of both upper thoracic chains prevents the liberation of adrenin not only from the adrenals, but also from most of the accessory chromaffine glands and possibly from the smooth muscle (Cannon and Bacq, 8). The decreased activity recorded during the 10 first days following adrenal inactivation is perhaps due to a temporary lack of cortical secretion. There is an unquestionable regeneration of the preganglionic fibres innervating the left adrenal, but it undoubtedly cannot occur in so short a time as 10 days.

The fact that rats with the sympathetic supply to the four limbs destroyed have approximately the same level of voluntary activity as their controls, demonstrates that by this criterion fatigue is not more readily induced after sympathectomy.

SUMMARY AND CONCLUSIONS

(1) In 10 albino rats, abdominal sympathectomy did not change the spontaneous activity as compared with that of controls.

(2) Abdominal sympathectomy and adrenal inactivation did not affect for more than 10 days the voluntary activity of 13 rats. During these 10 days, their activity was slightly below that of their controls.

(3) Subsequent removal of one or both stellate ganglia with the upper part of the thoracic sympathetic chains failed to have any significant effect on the spontaneous activity of 10 animals, as compared with controls.

(4) The enlargement of the seminal vesicles, which occurs in rats after abdominal sympathectomy, was not associated with any change in the activity level.

(5) It is concluded that removal of the sympathetic nerve supply to the genital organs and adrenals does not affect the normal secretion of the endocrine products which are known to affect the spontaneous activity of the rat. Sympathetic denervation of the four limbs does not reduce the muscular activity of the rat to the extent of decreasing the voluntary activity.

I am much indebted to Dr. M. O. Lee for many suggestions and frequent help during the progress of this work.

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ANTERIOR PITUITARY HORMONES*

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DETROIT

As our knowledge of endocrine glands increases it becomes more and more evident that the number of active principles is very much greater than had formerly been supposed. The separation of two hormones from the posterior lobe of the pituitary gland (1) stimulated speculation along this line but we must not allow ourselves to jump at the conclusion that each physiological action is controlled by a separate hormone.

In the case of the anterior lobe of the pituitary gland a rather complicated situation has resulted from the independent investigations carried on by numerous research workers. Experiments upon animals have shown eleven distinct activities of the anterior pituitary. These may be listed as follows: (I) stimulation of growth; (II) stimulation of sexual development and ripening of follicles, resulting in ovulation; (III) stimulation of lutein cells, resulting in prevention of ovulation by imprisoning the ova; (IV) stimulation of sexual development by a substance which can be given by mouth, in distinction to II, which is of very little effect when given by mouth; (V) stimulation of metabolism by increasing the specific dynamic action of food substances; (VI) stimulation of the thyroid gland; (VII) lowering of gaseous metabolism; (VIII) stimulation of water intake and output; (IX) stimulation of lactation; (X) lowering of non-protein nitrogen in blood; (XI) initiation of the bleeding of menstruation.

I. GROWTH HORMONE

The presence of the growth hormone was first recognized and demonstrated by Evans and Long in 1921 (2). They made saline suspensions of finely ground beef anterior pituitaries which were injected intraperitoneally into rats. By repeating this daily for several weeks, the rats were made to grow to be giants, twice the size of untreated controls. The next step was to prepare extracts by means of dilute alkali. Such extracts were neutralized with acid and centrifuged. A later improvement was sterilization by filtration through germ proof filters (3, 4, 5, 6).

A method suitable for the preparation of large quantities of extract containing the growth hormone was described by Putnam, Teel and Benedict in 1928 (7). A great improvement in the method, worked out by this group, was the concentration of globulins and active principle by means of salting out with sodium sulphate (8).

In our hands a slight modification of the method described by Teel (8) seems to yield the most potent preparations of the growth hormone.

*Read in part before the American Chemical Society, September 10, 1930, at Cincinnati, Ohio.

Beef pituitaries are chilled or frozen at the packing house as soon as practicable after the death of the animals. The anterior lobes are separated by dissection with a sharp knife and are ground very fine in a Latapie grinder. To every 100 grams of anterior lobes is added 400 cc. of 0.05 N sodium hydroxide. The mixture is stirred at intervals and then is allowed to stand in the refrigerator over night. It is neutralized to pH 7.2 to 7.6 by the addition of 0.2 N acetic acid. The insoluble gland particles are thrown out by centrifugation for half an hour. The cloudy supernatant liquid is heated to 37° C. on a water bath and to every 100 cc. is added 20 grams of sodium sulphate. A protein precipitate forms rather quickly. Centrifugation for half an hour separates the precipitate and supernatant liquid. The precipitate is then dissolved as far as possible in 0.02 N sodium hydroxide so that 2 cc. represents the extractives of 1 gram of anterior lobe. The gland residue, left after centrifugation of the first extract, is worked up in a similar way with one-half the volume of 0.05 N sodium hydroxide used in the first extract. After this second extract has been salted out with 20 per cent sodium sulphate, the precipitate is added to the solution of the first extract. A sterile product is obtained by filtration through asbestos and germ proof porcelain filters. This extract retains its potency for several months if kept in the refrigerator.

It seems probable that the filtration removes some of the growth hormone. That the sterile solution contains enough of the growth hormone to produce experimental acromegaly in dogs, has been reported by Putnam, Benedict and Teel (9). Accelerated growth of rats was reported by Putnam, Teel and Benedict (7) in confirmation of earlier experiments by

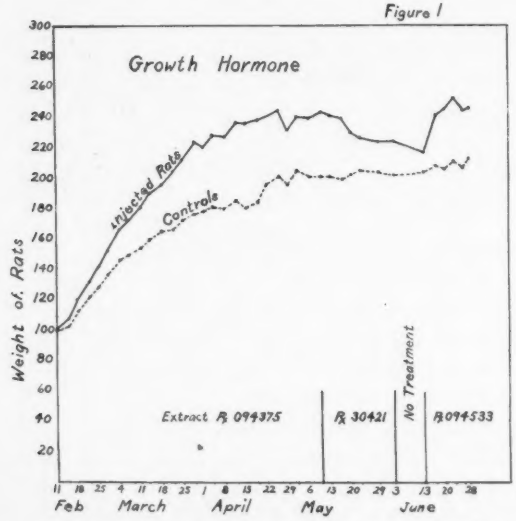

Figure 1. Average weight of 5 rats injected with growth hormone compared with 5 controls, showing stimulation of growth when active extracts were injected and loss of weight when an inactive extract was injected.

Evans and Long (2). This effect on rats has been demonstrated in several experiments in this laboratory. The data of a typical experiment are shown in Figure I. In the five treated rats injections were begun with doses of ¼ cc. subcutaneously daily, except Sundays. The dosage was increased every few days and when doses of over 1 cc. were reached, the injections were given intraperitoneally instead of subcutaneously. It will be noted that the treated rats continued to gain weight more rapidly than the controls from February 11th until May 10th. At this time they weighed 42 grams more than the controls. When an extract made by another method was substituted, the treated rats immediately began to lose weight. In a period when no treatment at all was given for 10 days, the rats still continued to lose weight. On June 13th injections were begun with an extract similar to the first and the rats immediately began to gain weight rapidly and in 10 days had increased 35 grams.

As far as our present knowledge goes, the growth hormone is entirely gland specific, that is, it has never been obtained from any source except anterior pituitaries.

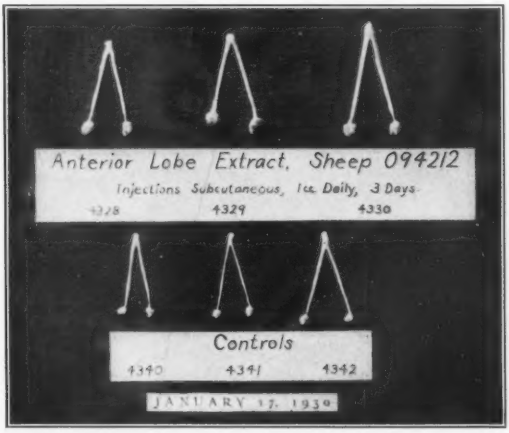

Figure 2. Uteri and ovaries of injected rats compared with controls. Alkaline extract of sheep pituitaries injected 3 days. For data see Table 2.

II, III. SEX HORMONES

The follicle stimulating hormone and the luteinizing hormone are often considered together as the "master sex hormone" or the "universal non-specific sex hormone," which is common to both sexes and which activates the gonads and all organs concerned with reproduction. It is the factor responsible for the sexual development which occurs at the age of puberty in both sexes.

At present we have no positive information as to whether there are really two sex hormones or whether only one sex hormone produces dif-

ferent effects under different conditions, particularly when supplemented by other hormones.

The universal sex hormone can be extracted from anterior pituitaries, placentas, amniotic fluid, blood of pregnant animals, but the richest source of all is the urine of pregnant animals. Very early in pregnancy large amounts of the sex hormone are excreted in the urine. This is the basis for the Aschheim-Zondek (10) test for pregnancy in women. A few drops of urine (0.2-0.4 cc.) are injected into immature female mice twice daily for 3 days. The mice are killed on the fifth day of the test, 100 hours after the first injection, and the ovaries are examined. Corpora lutea and follicles filled with blood are definite signs of the presence of the sex hormone. This is presumptive evidence that the urine was excreted by a pregnant woman. There are some other conditions such as chorioepithelioma which might give a false positive test and should be considered in a diagnosis (11).

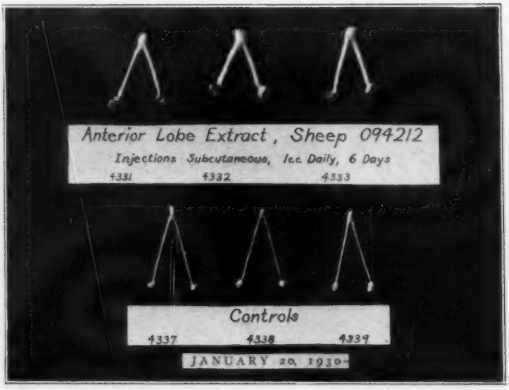

Figure 3. Uteri and ovaries of injected rats compared with controls. Alkaline extract of sheep pituitaries injected 6 days. For data see Table 3.

Evans and Simpson (12) evidently believe that there are two separate and distinct sex hormones, for they make the following statement: "Grounds can be found for the assumption that the stimulant to follicular growth is not identical with that causing luteinization."

Friedman (13) found that the intravenous injection of urine of pregnant women provokes ovulation in the rabbit while the intraperitoneal injection of similar urine produces luteinization of follicles without ovulation.

Smith and Engle (14) and Engle (15) have shown conclusively that anterior pituitary implants cause stimulation of follicular development with superovulation, whereas, injections of urine of pregnant women cause luteinization of follicle walls with prevention of ovulation due to the fact

that the ova are imprisoned within the rapidly developing wall of lutein cells.

Aschheim and Zondek claim to have obtained the two hormones separately by preparing them from different sources (16, 17, 18, 19). The follicle stimulating hormone, which Zondek has named Prolan A, was extracted from urine of women in the menopause and after ovariectomy. Such extracts when injected into mice were said to have produced only stimulation of follicles with ripening and ovulation. The luteinizing hormone, named Prolan B by Zondek, is said to have been prepared by Aschheim from anterior pituitaries. This extract produced luteinization of follicle walls so that corpora lutea formed quickly and imprisoned the ova.

Claus (20, 21) reported that she had separated the follicular ripening hormone from the luteinizing hormone and had isolated the former in crystalline form. Confirmation of this report has not yet been received.

Several articles in the literature by Evans and Simpson (5), Bellerby (22), Spaul (23) and Putnam (24), are responsible for the rather common belief that the sex hormones of the anterior pituitary are acid soluble in sharp distinction to the growth hormone which is soluble in alkali.

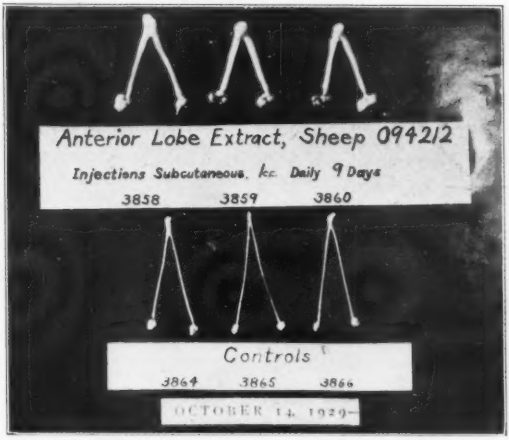

Figure 4. Uteri and ovaries of injected rats compared with controls. Alkaline extract of sheep pituitaries injected 9 days. For data see Table 4.

We have tried many times to make acid extracts of beef anterior lobes and whole sheep pituitaries. Such extracts have usually been inactive but a few have shown slight reactions characteristic of the sex hormones. On the other hand when we have extracted glands of the same lots with dilute alkali, we have obtained extracts which were many times as potent as those made with acid, judged by hypertrophy of ovaries of immature rats, following subcutaneous injections. Parkes (25) has reported upon the effectiveness of alkaline extracts of anterior pituitaries.

Alkaline Extraction of Sex Hormones

Dr. P. E. Smith, in a personal communication, furnished us with the information that sheep or hog pituitaries contain very much more sex hormone than beef pituitaries. In early work we attempted to dissect the anterior lobes away from the posterior lobes in sheep pituitaries but found it nearly impossible to make a clean separation. We tried extracting the entire gland and found that this gave extracts of apparently the same activity as those made from anterior lobes alone.

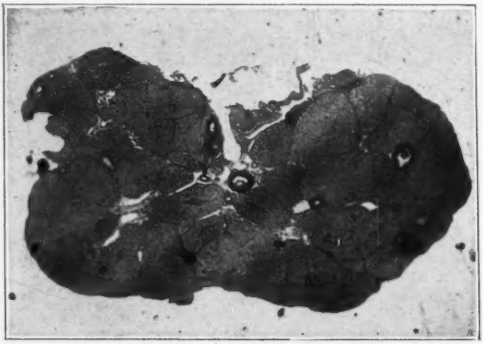

Figure 5. Section of ovaries of rat No. 3860 injected for 9 days with alkaline extract of sheep pituitaries. X18.

Our present method of preparing alkaline extracts for sex hormone experiments is quite similar to that given by Evans (4, 5), Teel (26), Putnam, Teel and Benedict (7), for the preparation of the growth hormone extract.

Fresh sheep pituitaries are frozen at the packing house and shipped in that condition. The glands are spread out and allowed to thaw at room temperature. They are immediately ground very fine in a Latapie grinder. To every 100 grams of ground pituitaries is added 400 cc. of 0.05 N sodium

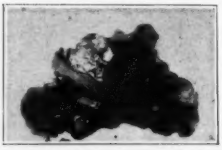

Figure 6. Section of ovaries of control rat No. 3863. X18.

hydroxide and 0.4 per cent tricresol. The mixture is stirred at frequent intervals for an hour. In some cases such mixtures have been allowed to stand in the laboratory for 2 days without any apparent injury. The extract is then neutralized to pH 7.2 to 7.6 (salmon pink to phenol red indicator) by the addition of N sulphuric acid. The extract may then be strained through cheese cloth. A still better procedure is to throw out the insoluble gland particles by centrifugation. This leaves a cloudy solution which may be injected readily through a fine hypodermic needle.

Such a solution is usually not sterile because it is contaminated by spores of hay bacillus. The tricresol is an effective preservative, however, and solutions containing 0.4 per cent have been kept for 6 months at room temperature with no evidence of putrefaction. There has been a loss of about 50 per cent in potency of the hormone which causes luteinization of the ovaries of immature rats. A second extract of the gland residue is made with half as much 0.05 N sodium hydroxide and 0.4 per cent tricresol as was used for the first extraction. After standing a few hours it is neutralized to pH 7.2 to 7.6 with N sulphuric acid. After centrifugation the supernatant liquid is similar to the first extract in appearance except that it is not as red. It is usually slightly more potent than the first extract.

A third extract may be made with one-quarter of the original volume of 0.05 N sodium hydroxide and 0.4 per cent tricresol. After neutralization and centrifugation this third extract will be found to be about the same potency as the first and second extracts.

The gland residue even after three extractions contains a considerable amount of the luteinizing sex hormone. It has not been determined how many extractions would be required to get all of this hormone out of the gland residue. Such extracts are effective in doses of 1 cc. daily for 3 days when injected subcutaneously into immature rats. When given for 6 or 9 days they cause hypertrophy of the ovaries to 5 to 10 times the size of ovaries of controls of the same age. This hypertrophy of the ovaries is shown in Figures 2, 3 and 4, and Tables 1, 2, 3 and 4. Tables 2 and 3 record data of experiments performed three months later than experiments recorded in Tables 1 and 4. The fact that the same extract was used with comparable results shows quite definitely that little if any deterioration had taken place in three months.

The microscopic appearance of the ovaries pictured in Figure 4 is shown in Figure 5. When these ovaries are compared with those of controls of the same age, Figure 6 (same magnification), it is quite apparent that injections of the alkaline extract of sheep pituitaries has caused tremendous development of the lutein cells of the ovaries.

IV. MATURITY HORMONE (COLLIP) EFFECTIVE BY MOUTH

Collip (27, 28) has recently reported the preparation from the placenta of a sex hormone, Emmenin, which produces precocious sexual maturity in young rats when given by mouth. About 0.01 mgm. of Collip's "micro-crystalline" substance is the effective dose for a rat. Zondek (29) had previously reported that the anterior pituitary sex hormone (Prolan) was effective when given by mouth to rats but not effective in mice. Doubtful results had been obtained when Prolan was given by mouth to women. At the present stage of our knowledge regarding Emmenin, it is not clear whether Collip has obtained Zondek's Prolan or has discovered a previously unknown anterior pituitary hormone or a hormone elaborated by some other organ, as for example, the placenta.

TABLE 1

ANTERIOR PITUITARY EXTRACT, SHEEP, 094212
Three Daily Subcutaneous Injections, 1 cc. Each

Rats	October, 1929					Vagina	Uterus and Ovaries	Ovaries	Corpora Lutea
	3	4	5	6	7				
3855	1 cc. 50 gm.	1 cc.	1 cc.	....	59 gm.	Partly open	80 mgm.	40 mgm.	Many
3856	1 cc. 50 gm.	1 cc.	1 cc.	....	62 gm.	Open	130 mgm.	70 mgm.	Many
3857	1 cc. 50 gm.	1 cc.	1 cc.	....	66 gm.	Open	120 mgm.	70 mgm.	Many
	Controls								
3861	50 gm.	....	....	....	56 gm.	Closed	40 mgm.	15-20 mgm.	None
3862	52 gm.	....	....	....	64 gm.	Closed	75 mgm.	15-20 mgm.	None
3863	54 gm.	....	....	....	67 gm.	Closed	50 mgm.	15-20 mgm.	None

TABLE 2

ANTERIOR PITUITARY EXTRACT, SHEEP, 094212
Three Daily Subcutaneous Injections, 1 cc. Each

Rats	January, 1930												Vagina	Uterus and Ovaries	Ovaries	Corpora Lutea
	6	7	8	9	10	11	12	13	14	15	16	17				
4328	64 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	....	95 gm.	Partly open	140 mgm.	60 mgm.	Many
4329	68 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	....	108 gm.	Partly open	200 mgm.	65 mgm.	Many
4330	46 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	....	62 gm.	Closed	205 mgm.	80 mgm.	Many
	Controls															
4340	70 gm.	....	....	....	....	....	....	....	....	....	....	105 gm.	Closed	70 mgm.	20 mgm.	None
4341	60 gm.	....	....	....	....	....	....	....	....	....	....	86 gm.	Closed	50 mgm.	15 mgm.	None
4342	70 gm.	....	....	....	....	....	....	....	....	....	....	102 gm.	Closed	80 mgm.	30 mgm.	None

TABLE 3
Anterior Pituitary Extract, Sheep, 094212
Six Daily Subcutaneous Injections, 1 cc. Each

Rats	January, 1930															Vagina	Uterus and Ovaries	Ovaries	Corpora Lutea
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20				
4331	44 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	62 gm.	Partly open	190 mgm.	70 mgm.	Many
4332	50 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	88 gm.	Partly open	350 mgm.	140 mgm.	Many
4333	58 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	98 gm.	Partly open	250 mgm.	75 mgm.	Many
	Controls																		
4337	48 gm.	....	....	....	....	....	....	....	....	....	....	....	....	....	75 gm.	Closed	*25 mgm.	15 mgm.	None
4338	56 gm.	....	....	....	....	....	....	....	....	....	....	....	....	....	95 gm.	Closed	25 mgm.	15 mgm.	None
4339	50 gm.	....	....	....	....	....	....	....	....	....	....	....	....	....	85 gm.	Closed	25 mgm.	15 mgm.	None

*Weights of uteri and ovaries are averages after partially dried.

TABLE 4
Anterior Pituitary Extract, Sheep, 094212
Nine Daily Subcutaneous Injections, 1 cc. Each

Rats	October, 1929												Vagina	Uterus and Ovaries	Ovaries	Corpora Lutea
	3	4	5	6	7	8	9	10	11	12	13	14				
3858	1 cc. 50 gm.	1 cc.	1 cc.	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	80 gm.	Open	380 mgm.	90 mgm.	Many
3859	1 cc. 50 gm.	1 cc.	1 cc.	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	78 gm.	Open	400 mgm.	160 mgm.	Many
3860	1 cc. 50 gm.	1 cc.	1 cc.	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	90 gm.	Open	370 mgm.	160 mgm.	Many
	Controls															
3864	50 gm.	....	....	....	....	....	....	....	....	....	....	80 gm.	Closed	50 mgm.	15-20 mgm.	None
3865	50 gm.	....	....	....	....	....	....	....	....	....	....	80 gm.	Closed	25 mgm.	15-20 mgm.	None
3866	52 gm.	....	....	....	....	....	....	....	....	....	....	84 gm.	Closed	35 mgm.	15-20 mgm.	None

TABLE 1

ANTERIOR PITUITARY EXTRACT, SHEEP, 094212
Three Daily Subcutaneous Injections, 1 cc. Each

Rats	October, 1929					Vagina	Uterus and Ovaries	Ovaries	Corpora Lutea
	3	4	5	6	7				
3855	1 cc. 50 gm.	1 cc.	1 cc.	....	59 gm.	Partly open	80 mgm.	40 mgm.	Many
3856	1 cc. 50 gm.	1 cc.	1 cc.	....	62 gm.	Open	130 mgm.	70 mgm.	Many
3857	1 cc. 50 gm.	1 cc.	1 cc.	....	66 gm.	Open	120 mgm.	70 mgm.	Many
	Controls								
3861	50 gm.	....	....	....	56 gm.	Closed	40 mgm.	15-20 mgm.	None
3862	52 gm.	....	....	....	64 gm.	Closed	75 mgm.	15-20 mgm.	None
3863	54 gm.	....	....	....	67 gm.	Closed	50 mgm.	15-20 mgm.	None

TABLE 2

ANTERIOR PITUITARY EXTRACT, SHEEP, 094212
Three Daily Subcutaneous Injections, 1 cc. Each

Rats	January, 1930												Vagina	Uterus and Ovaries	Ovaries	Corpora Lutea
	6	7	8	9	10	11	12	13	14	15	16	17				
4328	64 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	....	95 gm.	Partly open	140 mgm.	60 mgm.	Many
4329	68 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	....	108 gm.	Partly open	200 mgm.	65 mgm.	Many
4330	46 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	....	62 gm.	Closed	205 mgm.	80 mgm.	Many
	Controls															
4340	70 gm.	....	....	....	....	....	....	....	....	....	....	105 gm.	Closed	70 mgm.	20 mgm.	None
4341	60 gm.	....	....	....	....	....	....	....	....	....	....	86 gm.	Closed	50 mgm.	15 mgm.	None
4342	70 gm.	....	....	....	....	....	....	....	....	....	....	102 gm.	Closed	80 mgm.	30 mgm.	None

TABLE 3
Anterior Pituitary Extract, Sheep, 094212
Six Daily Subcutaneous Injections, 1 cc. Each

Rats	January, 1930															Vagina	Uterus and Ovaries	Ovaries	Corpora Lutea
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20				
4331	44 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	62 gm.	Partly open	190 mgm.	70 mgm.	Many
4332	50 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	88 gm.	Partly open	350 mgm.	140 mgm.	Many
4333	58 gm.	....	....	....	....	....	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	98 gm.	Partly open	250 mgm.	75 mgm.	Many
	Controls																		
4337	48 gm.	....	....	....	....	....	....	....	....	....	....	....	....	....	75 gm.	Closed	*25 mgm.	15 mgm.	None
4338	56 gm.	....	....	....	....	....	....	....	....	....	....	....	....	....	95 gm.	Closed	25 mgm.	15 mgm.	None
4339	50 gm.	....	....	....	....	....	....	....	....	....	....	....	....	....	85 gm.	Closed	25 mgm.	15 mgm.	None

*Weights of uteri and ovaries are averages after partially dried.

TABLE 4
Anterior Pituitary Extract, Sheep, 094212
Nine Daily Subcutaneous Injections, 1 cc. Each

Rats	October, 1929												Vagina	Uterus and Ovaries	Ovaries	Corpora Lutea
	3	4	5	6	7	8	9	10	11	12	13	14				
3858	1 cc. 50 gm.	1 cc.	1 cc.	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	80 gm.	Open	380 mgm.	90 mgm.	Many
3859	1 cc. 50 gm.	1 cc.	1 cc.	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	78 gm.	Open	400 mgm.	160 mgm.	Many
3860	1 cc. 50 gm.	1 cc.	1 cc.	....	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	....	90 gm.	Open	370 mgm.	160 mgm.	Many
	Controls															
3864	50 gm.	....	....	....	....	....	....	....	....	....	....	80 gm.	Closed	50 mgm.	15-20 mgm.	None
3865	50 gm.	....	....	....	....	....	....	....	....	....	....	80 gm.	Closed	25 mgm.	15-20 mgm.	None
3866	52 gm.	....	....	....	....	....	....	....	....	....	....	84 gm.	Closed	35 mgm.	15-20 mgm.	None

V. SPECIFIC DYNAMIC ACTION

It has been asserted by Kestner, Liebeschütz-Plaut and Schadow (30) that there is an anterior pituitary hormone which is responsible for the specific dynamic action of food substances. The German investigators claim that in the absence of this hormone the obesity of dystrophia adiposogenitalis occurs. Foster and Smith (31) have investigated this by means of careful basal metabolism experiments with rats. Injections of glycocoll were given to observe the specific dynamic action. These American investigators found that operative removal of the whole pituitary gland did *not* cause obesity but that brain injury in the region of the tuber cinereum did cause obesity. Removal of the whole pituitary caused loss of specific dynamic action. This loss was not overcome by replacement therapy by means of transplantation of anterior lobes or posterior lobes separately. When both lobes were transplanted the specific dynamic action of glycocoll injections was restored.

When only the posterior pituitary was ablated, the level of metabolism was not lowered but the specific dynamic action of glycocoll was abolished. When injections of bovine posterior pituitary extract were given daily for 10 to 14 days, the deficiency was overcome and glycocoll again showed a specific dynamic action.

VI. STIMULATION OF THYROID

Foster and Smith (31, 32) found that removal of the whole pituitary glands in rats caused a 35 per cent reduction in the basal metabolic rate. This was restored to normal by daily transplantation of anterior pituitaries. The transplantation of posterior pituitaries did not affect the basal metabolic rate. Administration of thyroid was effective, however. These investigators observed atrophy of the thyroid in the rats from which the pituitaries were removed. Daily transplants of fresh pituitaries caused repair of the thyroid gland. This evidence together with that of other investigators, Schwartzbach and Uhlenhuth (33), Crew and Wiesner (34), leads to the belief that one of the hormones of the anterior pituitary activates the thyroid gland.

VII. LOWERING OF GASEOUS METABOLISM

Lee and Gagnon (35) have reported that daily injection of anterior pituitary extracts, which caused increased growth in rats, also caused a decrease in the metabolic rate. It is possible that the growth hormone itself may stimulate anabolism and at the same time inhibit catabolism. The sum total of the effect may thus be storing up of material in a manner requiring less oxygen and consequently registering lower gaseous metabolism.

VIII. STIMULATION OF WATER INTAKE AND OUTPUT

Teel (36) observed that dogs which were given daily injections of a preparation containing the growth hormone showed greatly increased water intake and output. In one animal the urinary excretion was 700 cc.

in 24 hours during a control period and 7300 cc. after injections were given. In our own laboratory the increase in water metabolism has also been observed but in a manner which suggested the possibility of a non-specific action rather than that of a diuretic hormone peculiar to the anterior pituitary.

IX. STIMULATION OF LACTATION

Frei, Stricker and Grueter (37, 38) have observed increased development of the mammary glands with abundant lactation when anterior pituitary extracts are injected into animals. These investigators believe, however, that the anterior pituitary hormone can cause lactation only in mammary glands which have been previously acted upon by a corpus luteum hormone. Putnam, Benedict and Teel (24, 9) observed secretion of colostrum in a female dog in which experimental acromegaly had been produced by injections of alkaline extracts of beef anterior pituitaries. No corpora lutea were found in the ovaries upon post mortem examination. Evans and Simpson (39, 40) observed hyperplasia of mammary apparatus and lactation in adult virgin rats after long continued injection with alkaline extracts rich in growth hormone. Parkes (41) produced scanty lactation in rabbits by injection of alkaline extracts of beef anterior pituitaries and came to the following conclusions: "Control injections into two males and two ovariotomized females gave no results whatever. It is thus clear that the anterior pituitary substance functions entirely through its effect on the ovary, and almost certainly through the luteal tissue produced." "The breakdown changes in the gland which occur when the building-up luteal stimulus is withdrawn appear to initiate lactation."

Recently Corner (42) has obtained additional evidence which seems to indicate that the corpus luteum is not essential for lactation but that one of the anterior pituitary hormones, acting alone, is able to cause the secretion of milk. Nelson and Pfiffner (43) have found by experiments with guinea-pigs that the anterior pituitary hormone can cause lactation without the aid of the corpus luteum. They have drawn the conclusion that some ovarian hormone must act upon the mammary glands previously to enable the anterior pituitary hormone to produce lactation.

X. DECREASE OF NON-PROTEIN NITROGEN IN BLOOD

Teel and Watkins (44) have demonstrated by careful study of the blood chemistry of dogs that there is a marked drop in the non-protein nitrogen following the injection of alkaline extracts of beef anterior pituitaries. Extracts which caused acromegalic growth in dogs and excessive growth in adult rats produced the fall in non-protein nitrogen more effectively than any control extracts not containing the growth hormone. The conclusion is drawn that the growth hormone mobilizes the non-protein nitrogen for the purpose of building up new protoplasm. The nature of the extracts used by Teel and Watkins does not rule out the possibility of active principles, other than the growth hormone, playing some rôle in the control of non-protein nitrogen in the blood.

XI. INITIATION OF BLEEDING OF MENSTRUATION

Hartman, Firor and Geiling (45) have discovered that the bleeding of menstruation in monkeys can be initiated by implantation of anterior pituitaries and by injections of extracts of anterior pituitaries or urine of pregnancy. Uterine bleeding resulted from injections of ovarian follicular hormone because of the stimulation of the animal's own pituitary gland. Previous hypophysectomy prevented the uterine bleeding except when replacement therapy was instituted by implants or extracts which supplied the missing anterior pituitary hormone. This brilliant piece of research supplies positive evidence that menstrual bleeding is due to the presence of an anterior pituitary hormone rather than due to the absence of a corpus luteum hormone.

CHEMICAL PROPERTIES OF HORMONES

The consideration of these eleven physiological activities of the anterior pituitary has not convinced us that there are eleven separate hormones present in this gland. One of these activities (IV, stimulation of sexual development by a substance which is effective when given by mouth) was demonstrated by means of an extract made from placenta and consequently may not be due to an anterior pituitary principle but rather to one produced in the placenta itself.

The other ten activities have been demonstrated by transplanting anterior pituitaries or by injecting alkaline extracts. It is probable then that all the hormones have many chemical properties in common. At present only two hormones have been distinguished by specific chemical reactions. These two are the growth hormone and a sex hormone responsible for luteinization. The marked difference between them, as noted in our laboratory, is that the sex hormone is not injured by 0.4 per cent tricresol, while the growth hormone is quickly and completely destroyed by this strength of preservative. This fact can also be deduced from a study of the literature for Putnam, Teel and Benedict (7) reported that phenol inactivated their growth hormone extracts, while Aschheim and Zondek (10) use 1 drop of tricresol to preserve 30 cc. of urine in their pregnancy test, which depends upon the presence of the anterior pituitary sex hormone.

We have found that the sex hormone can be desiccated and kept in that condition for at least 8 months without any loss of potency. Van Dyke and Wallen-Lawrence (46) also have reported that the growth hormone can be preserved by desiccation.

Both hormones can be salted out from glandular extracts by means of 20 per cent sodium sulphate. This may indicate that both hormones are adsorbed by proteins in a similar manner, though Evans, Smith and associates (47) reported that a separation could be obtained with 50 per cent ethyl alcohol, the sex hormone remaining in solution while the growth hormone was carried down by the precipitate or destroyed.

Both hormones can be kept for many days in weak alkali but are

destroyed rather quickly by weak acids. Both hormones are destroyed by slightly elevated temperatures and the extent of destruction is determined by the duration of exposure to heat.

At present we are engaged in a study of the chemical and biological properties of the active principles and will present more details in a future paper.

SUMMARY

There are eleven distinct physiological functions claimed by various research workers, for the anterior pituitary: (I) stimulation of growth; (II) stimulation of sexual development and ripening of follicles resulting in ovulation; (III) stimulation of lutein cell development, resulting in prevention of ovulation by imprisoning the ova; (IV) stimulation of sexual development by a substance which can be given by mouth; (V) stimulation of metabolism by increasing the specific dynamic action of food substances; (VI) stimulation of the thyroid gland; (VII) lowering of gaseous metabolism; (VIII) stimulation of water intake and output; (IX) stimulation of lactation; (X) lowering of non-protein nitrogen in blood; (XI) initiation of the bleeding of menstruation.

One of these activities (IV) may be due to a placental hormone rather than one elaborated by the anterior pituitary.

Ten of these activities have been demonstrated by means of transplants of anterior pituitaries or by alkaline extracts.

The hormones responsible for the ten activities are similar in their general chemical properties, such as stability to acid, alkali and heat.

A growth hormone is clearly separate from one causing luteinization in ovaries for the former is readily destroyed by 0.4 per cent tricresol, while the latter is not destroyed by the same concentration of tricresol.

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ACTIVITY OF GOLDFISH ON TESTICULAR SUBSTANCE DIET

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It had been observed that goldfish, when fed finely ground-up whole testicular substance of the ram, showed considerably more activity than when fed ordinary ground-up dried shrimp or other food. In order to demonstrate this graphically the apparatus (Figure I) described below was devised.

In Figure I, *A* is a glass tray, size 12x18 cm. *B* is a piece of ordinary printers' brass rule bent at right angles around the tray and projecting upward as shown in sketch. *C* is a wooden piece ½ cm. square carrying pieces of capillary glass tubing. *D* are tubes spaced at equal distances apart. These distances are slightly greater than the width of the body of the fish. *C* and *D* as a unit are arranged to swing freely between the fine steel pivot points *E* and *E*. An extension of the middle capillary tube forms the arm *G*, which in operation moves the recording needle *H*, causing it to register the movements of the fish on the smoked paper of the kymograph *I*. *J* is a guide post to keep the recording needle in correct position. Movements and method of recording are illustrated by the dotted outlines.

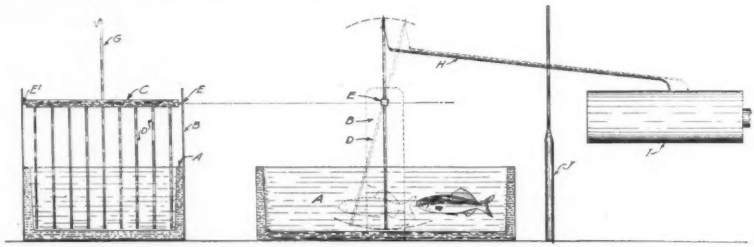

Fig. 1. Diagram of apparatus used to record movements of gold fish.

PROCEDURE

Three goldfish were placed in the tray and fed 1 gram of dried shrimp meat finely ground up. Their activities are recorded in tracings on Figure II.

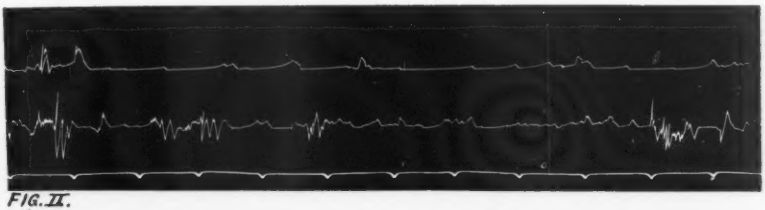

Fig. 2. Record of movements of fish when fed on dried shrimp meat.

The evenly spaced line represents fifteen-minute intervals.

At the end of twenty-four hours they were fed 1 gram of whole fresh ground-up testicular substance from the ram. Their activities are shown in the tracings of Figure III.

Fig. 3. Record of movements of fish when fed on macerated testicular substance of ram.

The evenly spaced line represents fifteen-minute intervals.

The circumstances of both these tests were the same, the fish being in ordinary artificial light away from any excitants, such as noise or motion.

It is seen that with shrimp food the fish in 24 hours made 1648 movements. With the testicular food during an equal period they made 6828 movements, an increase of 400 per cent.

These tests were repeated three different times with approximately the same results.

COMMENT

It is difficult to draw conclusions of any practical value from these experiments. This paper is submitted merely as an addition to the data of endocrinology.

ENDOCRINE GLANDS AND THE TEETH

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While a great deal is known about infected teeth and their relation to arthritis, gall bladder disease, gastric ulcer, etc., very little is known about the effect of infected teeth on the endocrine system; yet the importance of this subject can hardly be over-estimated. That there should be a close relationship between the endocrine system and the teeth is to be expected from our knowledge of the rôle played by the endocrines in calcium metabolism, osseous growth and immunity (1). The sensitiveness of the endocrine glands to toxins in other infections is also well known; for example, the damaged testicles which frequently occur in mumps and the occasionally injured adrenals in diphtheria. The chronic infections which occur at the apices of the teeth in the form of granulomata, blind abscesses, osteomyelitis, etc., frequently exist for a great number of years with the continuous absorption of the bacteria and their toxins in the circulation. This results in a condition particularly conducive to the damage of the endocrines (2). This investigation was undertaken to test the truth of the principle propounded.

Over two hundred consecutive office patients suffering from the various endocrine diseases enumerated below form the basis of this work. These were white people, practically all adults, and about twice as many females as males. Every tooth in the head was carefully examined, those missing charted and the condition of the rest of the teeth noted. Almost invariably it was found that if a person had many missing teeth the remaining teeth were in poor condition; conversely, if few teeth were missing the remaining ones were in much better shape. I believe that this is due not only to the fact that patients are loath to part with all their teeth (and even though badly diseased like to retain them as long as possible), but also where many teeth are extracted malocclusion results which leads to deterioration and infection of the remaining teeth. The teeth were lost spontaneously or extracted on account of disease. Whatever the cause of the primary dental disease may have been—disturbance of the calcium and phosphorus metabolism, lack of vitamin, constitutional disease, dental appliances, etc.—the final condition resulted in an infected tooth. Therefore, every missing tooth can be regarded as an infected tooth before it was lost and a potential etiological factor for endocrine and other diseases.

Needless to say, every effort was made to establish the correct diagnosis as far as was possible. This included not only a careful history, physical examination, a complete laboratory investigation of the blood, urine and sputum, but also, wherever indicated, the patients were sub-

mitted to fluoroscopy, x-ray examination and to a basal metabolism determination. A Wassermann test was made on every patient. Search was made for other foci of infection aside from the teeth. Sometimes infection was found in the tonsils, sinuses, cervix uteri, etc. Wherever possible such foci were eradicated and autogenous vaccine immunization instituted. Operations confirmed the diagnoses on some patients. No patient consulted me about the teeth. Very few were ultimately labeled with only one diagnosis. By far the great majority suffered from multiple diseases.

The missing teeth recorded in our table are classified under each one of the diseases. For example, a patient suffering from diabetes, obesity and goiter has a certain five teeth missing. These five teeth are recorded not only under diabetes but under obesity and goiter as well. While this method may lead to puzzling results in a few of the complications, apparently yielding a false relationship between such complications and the teeth, in the great majority of such instances the results fully corroborate our present known existing relationship and, in not a few cases, point to a more plausible revision of our present knowledge, linking certain endocrine diseases with bad teeth. For instance, we find that there is a great loss of teeth in obesity. On superficial observation this is hard to explain. However, we know how frequently obesity precedes and co-exists with diabetes; the loss of teeth in diabetes is well known. Diabetes is furthermore caused by disease of the endocrine system, viz., the islands of Langerhans. It is therefore logical to reason that the great loss of teeth in obesity has the same explanation as in diabetes. I will try to show later in this paper that it is the toxins from infections which damage the endocrines, resulting in the disease in question. It is the illumination of puzzling and obscure facts about certain endocrine diseases by new information of this nature that justifies such a classification and the publication of this paper itself. For the sake of brevity and clearness the subject matter is reduced as much as possible to table form.

DIABETES MELLITUS

Thirteen patients in this group lost more than half of their teeth (56¼ per cent). The loss was fairly equally divided between the upper and lower jaws, one hundred and twenty-two and one hundred respectively. The numerical losses in both jaws were in the following order: molars, bicuspids, central incisors, lateral incisors and canines. This systematic loss of teeth as to number, kind and sequence is certainly very striking and occurs in every one of the diseases mentioned below. The teeth are not lost haphazardly but in a definite order. The loss of teeth is so frequent that it forms a conspicuous objective sign of this disease. It very often precedes the diabetes and is almost invariably the first sign of the disease. The patients are first seen by the dentist and not by the doctor. It behooves every dentist to make a urine examination for sugar in patients with unexplainable loss of teeth. Better still, every dentist should be a physician. The explanation for this tremendous loss of teeth

TABLE I

MISSING TEETH IN NORMAL PERSONS

Age	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	
MALES													
No. Cases	4	1	12	11	3	4	6	2	2	1	1	1	48
Upper Jaw—													
Central Incisor	0	0	3	0	0	4	2	0	0	2	0	2	13
Lateral Incisor	0	0	2	2	0	4	2	0	0	2	0	2	14
Canine	0	0	0	0	1	4	2	1	0	2	0	2	12
Bicuspid	0	0	2	2	0	7	4	1	2	4	1	4	27
Molar	0	0	9	6	2	16	9	0	7	6	2	6	63
Lower Jaw—													
Central Incisor	0	0	0	0	0	4	4	0	0	0	0	2	10
Lateral Incisor	0	0	0	0	0	4	4	1	0	0	0	2	11
Canine	0	0	0	0	0	4	4	0	0	0	0	2	10
Bicuspid	0	0	0	0	1	8	10	0	0	0	0	4	23
Molar	0	0	9	5	2	12	15	0	5	3	2	6	59
Total Missing	0	0	25	15	6	67	56	3	14	19	5	32	242
Average per Person	0	0	2.1	1.4	2	16.8	9.3	1.5	7	19	5	32	5.0
FEMALES													
No. Cases	11	8	3	6	9	6	4	3	1	0	1	0	52
Upper Jaw—													
Central Incisor	2	2	0	0	5	6	3	2	0	0	0	0	20
Lateral Incisor	2	2	0	0	4	6	5	2	0	0	0	0	21
Canine	0	0	0	0	6	4	4	2	0	0	2	0	18
Bicuspid	1	0	0	0	11	12	9	5	1	0	0	0	39
Molars	1	2	3	0	23	22	15	15	0	0	0	0	81
Lower Jaw—													
Central Incisor	0	0	0	0	0	4	2	2	0	0	0	0	8
Lateral Incisor	0	0	0	0	0	3	3	2	0	0	0	0	8
Canine	0	0	0	0	1	4	2	1	0	0	1	0	9
Bicuspid	3	0	0	0	6	8	5	3	0	0	1	0	26
Molar	0	2	5	2	29	16	13	9	3	0	1	0	80
Total Missing	9	8	8	2	85	85	61	43	4	0	5	0	310
Average per Person	.9	1.	2.6	.3	9.5	14.2	15.2	14.3	4	0	5	0	5.9
TOTAL CASES													
No. Cases	15	9	15	17	12	10	10	5	3	1	2	1	100
Upper Jaw—													
Central Incisor	2	2	3	0	5	10	5	2	0	2	0	2	33
Lateral Incisor	2	2	2	2	4	10	7	2	0	2	0	2	35
Canine	0	0	0	0	7	8	6	3	0	2	2	2	30
Bicuspid	1	0	2	2	11	19	13	6	3	4	1	4	66
Molar	1	2	12	6	25	38	24	15	7	6	2	6	144
Lower Jaw—													
Central Incisor	0	0	0	0	0	8	6	2	0	0	0	2	18
Lateral Incisor	0	0	0	0	0	7	7	3	0	0	0	2	19
Canine	0	0	0	0	1	8	6	1	0	0	1	2	19
Bicuspid	3	0	0	0	7	16	15	3	0	0	1	4	49
Molar	0	2	14	7	31	28	28	9	8	3	3	6	139
Total Missing	9	8	33	17	91	152	117	46	18	19	10	32	552
Average per Person	.6	.9	2.2	1.0	7.6	15.2	11.7	9.2	6	19	5	32	5.5

	No. Cases	No. Missing Teeth	Per Cent Missing Teeth
Males	48	242	15.8%
Females	52	310	18.7%
Both Sexes	100	552	17.6%

TABLE II
ENDOCRINE GLANDS AND THE TEETH

	Upper Jaw							Lower Jaw								
	No. of Cases	Central Incisor	Lateral Incisor	Canine	Bicuspid	Molar	Total Upper Jaw	Central Incisor	Lateral Incisor	Canine	Bicuspid	Molar	Total Lower Jaw	Total Both Jaws	Average	Percent Missing
Diabetes Mellitus	13	17	16	13	30	46	122	15	12	12	29	52	120	242	18	56¼
Menopause	30	35	32	29	73	113	282	16	16	17	63	105	217	499	16	50
Obesity	66	46	54	42	127	202	471	31	30	30	96	197	384	855	12	37½
Endocrines	151	107	110	91	255	409	972	76	75	73	216	456	896	1868	12	38.6
Hyperthyroid	41	19	21	17	51	82	190	9	10	12	45	103	179	369	9	....
Hypothyroid	12	6	4	4	15	31	60	5	4	4	10	36	59	119	9	....
Dysthyroid	6	4	4	2	9	9	28	2	2	2	6	16	28	56	9	....
Exophthalmic goitre	14	8	8	8	18	29	71	3	3	4	14	32	56	127	9	....
Cystic thyroid	2	2	2	2	4	8	18	2	2	2	4	7	17	35	17	....
Froelich's syndrome	7	6	4	4	13	13	40	0	0	2	5	14	21	61	8	....
Cystic ovary	8	6	6	6	15	22	55	4	4	4	10	19	41	96	12	....
No axillary hair	19	24	24	21	46	70	185	20	21	16	44	81	182	367	19	....
Hirsutism	1	2	2	2	4	6	16	2	2	0	0	0	4	20	20	....
Vicarious menstruation	1	0	0	0	0	4	4	0	0	0	0	4	4	8	8	....
Amenorrhea	2	2	2	2	5	9	20	0	0	0	2	3	5	25	12	....
Dysmenorrhea	3	4	4	2	8	15	33	3	3	3	9	15	33	66	21	....
Endocrine, mixed types	35	24	29	21	67	111	252	26	24	24	67	126	267	519	14	....
Non-erupted teeth	19	6	1	1	10	14	32	2	2	0	21	42	67	99	5	15¾
Total (Excluding non-erupted teeth)	260															

MISSING TEETH IN SEQUENCE

Upper Jaw—		Lower Jaw—		Both Jaws—	
Molars	770	Molars	810	Molars	1580
Bicuspids	485	Bicuspids	404	Bicuspids	889
Lateral Incisor	212	Central Incisor	138	Lateral Incisor	345
Central Incisor	205	Lateral Incisor	133	Central Incisor	343
Canine	175	Canine	132	Canine	307
Totals	1847		1617		3464

Percent teeth missing 41.6%
Percent more teeth missing in upper jaw than lower jaw 14.2%
Non-erupted teeth have not been included in any of these figures.

in diabetes is variable. From a large experience with this disease I believe that the most plausible explanation is that there is a primary focus of infection in the teeth from which the toxins and the bacteria gain access to the circulation and damage the islands of Langerhans of the pancreas, this being responsible for the diabetes. My reason for this belief is that there is not only focal dental infection very frequently associated with diabetes, but that it frequently precedes this disease. Of course the primary focus of infection need not be in the teeth only—it may be, and frequently is, located in the gall bladder. Another reason for this theory is the striking results one frequently obtains in ameliorating and even completely curing the disease by the simple expedient of eradication of focal infection. This occurs not only in mild cases of diabetes but even in severe cases, not only in those of short duration but also in those of long duration. The following two cases are typical examples of many which have come under my observation and illustrate the point in question.

Case 1. L. S., thirty-eight years old, presented himself sixteen years ago at my office complaining of severe attacks of boils which were located all over his body. Repeated blood and urine examinations revealed moderate hyperglycemia and glycosuria, respectively. With the exception of thirty pounds overweight and the boils the physical examination gave negative findings. An infected tooth was removed. The boils were incised and drained and an autogenous vaccine (Staphylococcus pyogenes aureus) was administered, and he was placed on an anti-diabetic diet. The patient made a complete recovery. The blood and urine gradually returned to normal. The patient was eating everything. Five years ago he again showed traces of sugar in the urine and upon the extraction of another infected tooth the sugar promptly disappeared. The patient today is normal in every respect and eats everything.

Case 2. On February 7, 1930, the ambulance picked up D. K., sixty years of age, and brought him to the hospital. The patient vomited and was unconscious. He could be roused only slightly with the greatest difficulty, and pointed to his head as hurting him. He would immediately fall unconscious again. On physical examination we found a fairly well nourished old man, with an epithelioma of the forehead, a purulent discharge of the right ear, markedly infected teeth with numerous retained infected roots, and a muco-purulent discharge from the nose. The ocular fundi showed sclerosed blood vessels. The temperature on admission was 104, pulse 110, respiration 24, blood pressure 190/90. The leucocyte count was 12,200 with 83 per cent polymorphonuclears, 13 per cent small lymphocytes, 2 per cent large lymphocytes, 1 per cent eosinophiles, 1 per cent transitional cells. A catheterized urine revealed sugar, ketone bodies, a slight amount of albumin and numerous granular casts. A chemical analysis showed sugar 200, urea nitrogen 31, and creatinine 2.8 mgms. to 100 cc. of blood. Insulin and the usual anti-diabetic treatment employed in such cases was instituted; the patient rallied quickly. The next day the temperature became normal and the coma almost completely disappeared. The ear and nose were cleared up, all infected root stumps were eradicated, and autogenous streptococcus vaccine was administered. The insulin administered was gradually lessened and finally stopped entirely, although the patient was still kept on an anti-diabetic diet. The blood chemistry and the urine became entirely normal.

BLOOD CHEMISTRY

Date	Sugar	Urea Nitrogen	Creatinine
Feb. 10, 1930	142	35.0	2.5
Feb. 15, 1930	145	19.1	2.0
Feb. 17, 1930	120	16.5	2.0
Feb. 24, 1930	116	....	...
Mar. 3, 1930	109	....	...

The urine followed a course similar and corresponding to the blood. From February 28 until the day of discharge the patient was placed on a mixed, regular diet without once showing any abnormalities in the blood or urine.

The strikingly good results in both cases mentioned following so closely on the heels of eradication of infection, leads me to believe that this was an important etiological factor. I believe that the infection comes first, the diabetes afterwards, and that once the diabetes is established, a vicious cycle occurs. The hyperglycemia, ketosis and other toxins formed in diabetes (I believe that there are many poisons found present in diabetes which are as yet unknown to us) lower the resistance of the tissue and form a favorable pabulum for the luxuriant growth of bacteria about the teeth. Another reason why the teeth suffer in this disease is because there is not only a disturbance of the metabolism of carbohydrate, fat, and protein, but also of calcium.

MENOPAUSE

There were thirty women in this group who suffered from symptoms which could be attributed only to the menopause. Only half their teeth were left. More teeth were lost in the upper jaw than in the lower jaw. Age and previous pregnancies do not entirely account for this tremendous loss of teeth in some of these women. As shown in a previous communication (3), one hundred and eighty-four pregnant women lost forty-four per cent of their teeth as compared to sixty-nine non-pregnant women who lost, on the average, fourteen per cent of their teeth; furthermore, all the women who suffered from the menopause had not had previous pregnancies. It is possible that the explanation lies in the disturbance of the calcium metabolism and its destructive influence on the teeth due to the endocrine glandular imbalance which is present at the menopause (4).

OBESITY

Sixty-six obese patients of endocrine origin comprising this group lost over thirty-seven per cent of their teeth. Looking at the tables, one is impressed with the strikingly identical resemblance in the order of loss of teeth in obesity and diabetes, the only difference being that more teeth are lost in the latter.

Why should obese people lose over thirty-seven per cent of their teeth? I believe it is because the great majority of them are potential diabetics. The loss of teeth in diabetes is too well known to mention. Joslin (5) has ventured the assertion that diabetes is largely a penalty of obesity, and the greater the obesity the more likely is nature to enforce the penalty. Adams (6), of the Mayo Clinic, has shown that ninety-one per cent of the patients have been over-weight and that eighty-two and nine-tenths per cent were more than ten per cent over-weight before their diabetes began. There is little relevant difference between the sexes. Adams concludes that the unpreventable causes of obesity may be age, sex, heredity, previous disease and various disturbances of the endocrine glands, including those which take place at the menopause. In any event each succeeding complication of ascertainable facts only serves to emphasize anew that education regarding obesity should help in averting diabetes. The high

percentage of missing teeth in both obesity and diabetes is another link in the chain of evidence that both diseases are closely related. Whatever has been said under the head of diabetes applies here, with one exception: I do not know whether the infected teeth come first, their toxins damaging the endocrine glands responsible for the obesity, or the reverse, viz., that these patients not only have a marked disturbance in their fat but also in their calcium metabolism and that this is the cause for their loss of teeth. Perhaps both factors operate in a vicious cycle. No one has ever before drawn attention to any relationship existing between infected teeth and obesity, as has been done in diabetes, yet such a relationship does exist. Further observation of this fact is necessary before definite conclusions can be drawn.

ENDOCRINES

There were one hundred and fifty-one patients in this group and each one lost, on the average, forty-six per cent of his teeth. The teeth lost in each sub-group can readily be seen by consulting Table 2. A word about some of the members of the sub-groups. Those relating to the thyroid, ovary, and pituitary need no explanation. There were nineteen patients who had no axillary hair. This was their main objective endocrinological stigma. Invariably these patients have a short menstrual cycle. By this I mean they began to menstruate late, almost never before sixteen instead of at thirteen or fourteen years as demonstrated in a previous paper (7), and have their menopause early in life, usually in the early forties. They have a marked tendency to develop hypertension and diabetes. I believe that nearly all of them should be grouped under the gonado-thyrotrope group. In the six patients with menstrual disturbances care was taken to rule out any local uterine disease. Their trouble was of endocrine origin—gonado-thyro- or thyro-pituitary. I have repeatedly noted the tremendous loss of teeth in uterine and ovarian disease. In speaking to dentists they also confirm the accuracy of this observation. It is rather difficult to explain why disturbances in the endocrines should be associated with such a marked loss of teeth. As this group is fairly large, an explanation on the grounds of accidental finding, whatever that may mean, can be excluded. We must take this extensive loss of teeth as an index of infection. As previously noted, the underlying cause in practically every instance was infection, no matter whether the teeth were lost spontaneously or extracted artificially. I wonder whether the endocrine glands are damaged, to a much greater extent than we have heretofore supposed, by the bacteria and their toxins arising from this dental infection. The converse may be true. We know that certain constitutional endocrine stigmata, particularly familial in type, are associated with marked loss of hair. Likewise, may we not have certain endocrine disturbances associated with marked loss of teeth? It is interesting to note that both hair and the teeth have the same developmental origin, namely, the skin. A third possibility is that both factors operate in a vicious cycle. Finally,

whatever the explanation may be, it is interesting and important to note that there is a marked loss of teeth in endocrine disease.

UNERUPTED TEETH

The most striking feature in these cases is the marked endocrine stigmata which appear in every instance. While no one realizes better than I that the term endocrine disturbance depends not only upon the object described but on the describer as well—for one observer will find a person perfectly normal whereas another will find all sorts of disturbances—at the same time there exists a group of subjective and objective symptoms which can only be explained upon an endocrine basis. It is only on this basis that a rational explanation can be found for a cretin or a myxoedemic or the numerous intermediary stages, the giant and the dwarf, the beardless man and the bearded woman, the absence of axillary hair, the female distribution of hair in the male and the male distribution in the female, hirsutism, the masculine female and the effeminate male, and a marked variation from the normal in the basal metabolic rate. Thyroid disturbance is so marked in all our cases without exception—either hyperthyroid, hypothyroid or mixed (usually a physically enlarged thyroid is present) together with the abnormal basal metabolism and other thyroid stigmata—that one cannot help but associate the unerupted teeth with this thyroid disturbance, although this may be a compensatory process. The sella turcica, which was examined radiographically in a good many of our cases, was found to be normal. Not only somatic endocrine changes were present in these cases, but also physiological changes with varying types of marked menstrual disturbances. The endocrine relationship of allergy, hypertension, and arthritis which one meets so frequently in such cases have been pointed out (8).

Timme (9) believes that anomalous upper lateral incisors are indicative of a pluriglandular compensatory deficiency. None of these patients presented the syndromes described by Timme. The upper lateral incisors are the teeth presumed by Kaplan (10) "to be connected with the gonads and carry the stamp of ovarian or testicular abnormalities in their shape, size and implantation. The greater the deviation of the gonads from the normal, the more definite the markings in the dental apparatus. The lateral incisor as a marked gonadal defect may not have erupted at all. In the male, small stumpy lateral incisors bespeak impotence as on a physical basis." Where the material is so small it is impossible to draw definite conclusions. However, one cannot but note the marked menstrual disturbance, the metrorrhagia, dysmenorrhea, premature menopause, late onset of menstruation (sixteen years or over), the absence of axillary hair and the obesity—all symptoms associated with gonadal disturbance.

A history of mumps is not an infrequent finding in these patients. That mumps damage the gonads is a well known fact. However, one frequently observes diseased gonads without anomalous lateral incisors and

also the converse. Appendectomy in the female following mumps has frequently its true cause in the gonads. Many of our patients gave a history of appendectomy or appendicitis. Others attribute this anomaly to evolution on the basis that, since the upper lateral incisors are the least useful teeth, there is a tendency to their disappearance. Still others believe that this is of no significance, that it is only a curiosity. This explains nothing. Wheelon (11) does not seem to come to any definite conclusion, although one cannot help but see the marked endocrine stigmata in the five cases he describes.

Of five married women in this group three were pregnant and two were sterile, but the number is too small to draw definite conclusions. Dysmenorrhea, metrorrhagia, menstruation beginning at sixteen or over and the premature menopause at thirty-five or forty are frequent occurrences in these patients or in their immediate relatives. Since a local pathological basis can be excluded it leaves an endocrine basis as the only explanation. In some of the cases of this group it is interesting to note that the cough and dysmenorrhea were helped only by endocrine therapy. Cases of uterine hemorrhage in which curettage and radium applications failed, got well after the administration of thymus, mammary and placenta (12) which would tend to suggest a marked endocrine disturbance. One patient who had suffered for a whole year from unrelenting headaches, where all methods of treatment had failed, was completely cured by the removal of an upper lateral incisor, and remained well for the last four and a half years. Another patient who had typical epilepsy since childhood has remained free from attacks since four impacted teeth were removed. In the last two cases the impacted teeth evidently pressed upon the trifacial nerve which reflexly gave rise to the disease. Undoubtedly there are other diseases in which impacted teeth play a predominating rôle by the reflex irritation of the tri-facial nerve.

BACTERIOLOGY

The bacteria usually found in these foci of dental infections were mainly the various types of streptococci, viz., the viridens, hemolyticus longus and brevis. The Staphylococcus pyogenes aureus and the Pneumococcus were also found. Occasionally the Friedlander bacillus, the diphtheroid and the influenza bacillus were present. Autogenous vaccine immunization is found very efficacious in the treatment of the above mentioned diseases. Very often there will be almost immediately after the extraction of a tooth an aggravation of the endocrine disease in question instead of an amelioration. This is due to the added sensitization by the bacteria expressed in the circulation from the operative field. To avoid this, three or four injections of vaccine made from the micro-organism in question given prior to the dental extraction will be sufficient to immunize the patient so that the extraction can be performed without harm.

DISCUSSION

It is interesting to note that in both health and disease the teeth are not lost haphazardly but in a definite order. Numerically this sequence is molars, bicuspids, central incisors, lateral incisors and canines. The molars are the first to go. The canines are the last. I wonder whether this can be explained on an atavistic and anthropologic basis. In the scale of evolution of man carniverous teeth are the first to develop. The herbiverous teeth develop at a much later period. We know that those organs or structures that developed later in the evolution of man are the first to go as compared with those developed in a more remote period. We therefore would expect the grinders such as the molars and the bicuspids to be lost before the rest of the teeth. I also believe that in addition to the above explanation another factor operates—the character of the structure of these herbiverous teeth. They are full of corners, angles and grooves and are therefore more susceptible to retention of decaying matter followed by acid formation and infection which results finally in extraction of the tooth.

Another curious fact which holds good in normal healthy people as well as in diseased is that more teeth—fifteen per cent—are lost in the upper jaw than in the lower jaw. While we know that the vascular and nervous supply of the upper teeth is different from that of the lower, that the upper jaw is more cancellous and stationary, while the lower is movable, still I believe that there is another and unknown factor operating here which is responsible for this phenomenon. Can the strikingly definite orderly loss of teeth as demonstrated in every one of the diseases mentioned above as well as the greater dental loss of the upper jaw be explained on an endocrine basis? This would indicate an intimate relationship and control of the various endocrine glands and the various teeth. Kaplan's claims may be correct after all (10).

One hundred normal people lost seventeen per cent of their teeth. In three hundred and one consecutive office patients composed of one hundred and eight males and two hundred and fifty-three females suffering from internal medical diseases in general, there was lost on the average over thirty-one per cent of their teeth. In more than two hundred patients suffering from endocrine diseases there was an average loss of forty-one per cent of the teeth.

CONCLUSIONS

There is a definite relationship between the various endocrine diseases and the loss of teeth.

The marked loss of teeth in diabetes and obesity is another link in the chain of evidence that both diseases are related, perhaps by damage of the endocrines.

It is highly probable that the toxins and the bacteria from focal dental infections damage the endocrine glands which in turn set up a

vicious circle. The endocrine glands, on account of their influence on the calcium metabolism, are an important factor in the loss of teeth.

Infected teeth, which frequently precede diabetes, result in damage to the islands of Langerhans.

The eradication of focal dental infection is a valuable therapeutic procedure in diabetes.

The teeth are not lost haphazardly but in a definite sequence in health as well as in disease. The molars are the first to go, the canines the last.

Fourteen per cent more teeth are lost in the upper jaw than in the lower.

Unerupted teeth are frequently associated with marked endocrine stigmata.

The average office patient (my office is that of an internist) lost thirty-one per cent of their teeth. Those suffering from endocrine diseases lost on the average forty-six per cent of their teeth.

Various diseases arising from reflex nervous irritation, caused by pressure from impacted teeth, can be cured by the extraction of those teeth.

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THE EFFECT OF SOLUBLE STREPTOTOXINS ON THE THYROID GLANDS OF RABBITS*

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The rôle of infections as etiological factors in the causation of thyroid disorders has been the subject of study by various investigators for a number of years. Although much information has come to light as to the various influences of environment, diet, functional disorders, and the particular rôle of iodine, it has always been held that micro-organisms must be considered as influencing thyroid disorders if not actually causing them.

Halstead (1), while attempting to explain the hypertrophy occurring in the stumps of the thyroid glands of dogs upon which thyroidectomies had been done, suggested the possible rôle of intoxication from the infected wounds of these animals. This observation was based upon his own previous experiments and those of Munk, whom he quotes (2). Halstead later repeated the experiment under conditions of asepsis and variations in diet but was unable to obtain hyperplasia of the remaining gland. This he thought was suggestive of the possible influence of infections obtained in his experiment of 1888. Marine pointed out that his failure was probably due to the use of iodine on the skin and not to the absence of infection. Farrant (3) observed a marked hyperplasia of the thyroid in guinea pigs given minimum lethal doses of diphtheria toxin. His experiments led him to the conclusion that the hyperplasia of the thyroid in response to the administration of diphtheria toxin was due to its probable rôle in the production of antitoxin. Farrant (4) pointed out that the pathological changes seen in exophthalmic goiter could only be reproduced in part by feeding animals thyroid extract. He also observed that in a number of infectious diseases such an infantile diarrhea, measles, diphtheria, and whooping cough, hyperplasia similar to that seen in exophthalmic goiter occurred. In his experiments and observations with different infections and various types of diseases he concluded that thyroid changes were dependent upon the amount, virulence and duration of the toxemia. It is interesting to note that streptococci were given no place in causing hyperplasia. Billings (5) referred to the relationship of focal infection to thyrotoxicosis and reported cases cured by tonsillectomy. Burget (6) was unable to demonstrate any hyperplasia in the thyroids of cats following the injection of streptococci in the tonsils. Nor was he able to produce hypertrophy in the thyroids of cats fed upon the solution of feces from

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an exophthalmic goiter patient and from goiterous dogs. Harvies (7), while studying the influence of intestinal bacteria on the thyroid gland, concluded that exophthalmic goiter is due to the absorption of tryptophan from the intestines due to the absence of indole producers. Abel, et al. (8), hold the opposite view, namely, that tryptophan is essential to thyroid function.

Recently the rôle of infections upon thyroid irregularities has received more attention. Cole and Womack (9) have shown that the iodine content of the thyroid is diminished in animals suffering from infections and that the occurrence of hyperplasia probably indicates the activity of the thyroid in combating disease in general and acute infections in particular. These same authors (10) produce such evidence in animals by ligating the appendix (peritonitis), by injecting streptococci and straphylococci in the pleural cavities and also by subcutaneous injections of fecal material into the subcutaneous tissue. They later demonstrated that such hyperplasia could be inhibited by the oral administration of iodine (11).

There can be do doubt even though the results of experimentation in the past have been somewhat conflicting and incomplete, that in addition to the many agencies which directly or indirectly affect the rôle of the thyroid gland in its relation to metabolism, focal infections with their diffusing toxins play a rôle.

About the time that Cole and his co-workers were making their studies we became interested in the possible influence of streptococcic disease upon thyroid activity. We were especially interested in the influence of nasopharyngeal and sinus disease in stimulating changes in the thyroid. We were also interested in the specific influences of the toxins elaborated by such organisms directly upon thyroid tissue. It may be assumed that the impingement of bacterial toxins upon tissues generally would give rise to an increased reaction on the part of the thyroid to aid in the necessity of the metabolism increase for the production of defense substances. But the question as to whether the thyroid itself is especially irritable to certain bacterial toxins has not been completely determined. With this in view we undertook the following experiments.

The first problem which confronted us was the preparation of the streptotoxin. Knowing that laboratory animals are only slightly susceptible to streptococci, it was apparent that, before the toxin could be prepared, in order that it might show its true effects, the organisms must be acclimated to the animals used, and their virulency enhanced to the point where the animals were markedly susceptible. This was attempted in the following manner.

In our experiments, strains of streptococci were obtained from various sources, chiefly from cases of acute sinusitis, pharyngitis, mastoiditis, and tonsillitis. We were able to demonstrate pathogenicity for rabbits with only 8 of the strains. Of these only 6 produced soluble toxins. Three of these were the Alpha hemolytic streptococci. The other five were the Beta

hemolytic types. A 24-hour broth culture of the streptococci was injected into rabbits by way of the marginal ear vein. The following day 10.0 cc. of the heart's blood was taken, one-half of this being immediately injected into a second rabbit in the same manner, and the remainder cultured. Both blood agar plates and blood broth were used. If these cultures were positive the procedure was repeated daily until the animals showed signs of severe illness or died. The organism was finally re-isolated in pure culture from the last animal preparatory to obtaining the toxin.

The technique followed for the preparation of the toxin was according to the method described by Dick (12). A few alterations were made however. Our routine was as follows: (1) The organisms were cultured in plain broth with 0.1 per cent sterile blood added to them. (2) The tubes were incubated for 6 days. (3) The cultures were passed through filter paper until clear. (4) The filtrate was passed through a Berkfeld filter. (5) The filtrate was cultured to insure sterility. (6) The filtrate was finally placed in sterile glass bottles in the refrigerator after adding a drop of chloroform to each.

The use of phenol in the filtrate as advocated by the Dicks was eliminated, since we feared that its deleterious effect on tissue would complicate our findings. The potency of this solution was gauged by the intravenous injection of small amounts and by skin reactions. Prostration or definite erythema about the skin area injected were considered positive.

Certain difficulties were encountered during the preparation of the toxin. Many strains of organisms died out due to lack of pathogenicity for rabbits. Other organisms strongly pathogenic for rabbits were apparently not toxin producers.

In order that we might observe the effect of the toxin so prepared upon the thyroid gland directly, it was necessary to improvise a method whereby the toxin, in its full potency, could reach the thyroid gland, unmodified by the action of other body tissues. The route of injection chosen in most of the animals was by way of the arterial blood to the thyroid. Injections were made on one side into the superior thyroid artery by way of the common carotid artery. This vessel was isolated and clamped off temporarily just above the origin of the superior thyroid branch. The injection was made into the common carotid just below this point with a 27 gauge hypodermic needle, bent at an angle of approximately 45 degrees to aid its manipulation. Passing through the artery wall at an angle eliminated practically all hemorrhage as well as the necessity of ligating the artery after the injection was completed. In other animals the injection was made directly into the thyroid gland. The glands were prepared by the paraffin technic after fixation in Zenker's fluid. Only one of the six toxins produced gave definite results. The others showed changes in the thyroid indicating minor focal activity.

Our first series of animals were injected with the toxin prepared from a strain of strongly hemolyzing streptococci (Beta) obtained from a mas-

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No.	Material	Amount	Symptoms	Lobe	Time	Epithelial Hyperplasia	Epithelial Hypertrophy	Diminution of Colloid	Stroma	Dilatation of Blood Vessels	Dilatation of Lymph Vessels	Degree of Activity
S-1 R-1	Soluble hemolytic streptotoxin	4 M.	Right eye bulging. Right palpebral fissure dilated	Right	24 hrs.	+	+++	Marked	Negative	Engorgement marked	Moderate	+++
				Left	48 hrs.	++	++	Moderate	Edema	Engorgement slight	Marked	++
S-1 R-4	Soluble hemolytic streptotoxin	2 M.	Death—70 hours. Post-operative septicemia	Right	48 hrs.	+	++	Slight	Slight increase. Edema—slight. Mono-nuclear phagocytes	Engorgement slight	Moderate	++
			Localized abscess Broncho pneumonia	Left	70 hrs.	0	+	Slight	Slight increase. Edema—slight. Mono-nuclear phagocytes	Engorgement extreme	Slight	+
S-1 R-2	Soluble hemolytic streptotoxin	2 M.	None	Right	12 hrs.	+	++++	Marked	Edema	Engorgement moderate	Narrow	+++
			None	Left	24 hrs.	+	+++	Marked	Edema	Engorgement slight	Slight	+++
S-1 R-3	Soluble hemolytic streptotoxin	2 M.	Right eye bulging before removal of first gland	Right	48 hrs.	++	+++	Marked	Slight increase endothelial phagocytes. Small coll. red cells	Engorgement slight	Slight	+++
			Right palpebral fissure dilated	Left	96 hrs.	++	++++	Moderate	Slight increase endothelial phagocytes. Small coll. red cells	Engorgement slight	Narrow	+++
S-1 R-6	Soluble hemolytic streptotoxin	2 M.	None	Right	192 hrs.	++	+	No	Edema ++ Mononuclear phagocytes—plasma cells. Pseudo lymph N.	Engorgement negative	Moderate	+
			None	Left	312 hrs.	++	+	Moderate	Edema—Mononuclear phagocytes beneath capsule	Engorgement slight	Marked	+
S-1 R-7	Soluble hemolytic streptotoxin	2 M.	None	Right	336 hrs.	0	0	No	Edema. Mononuclear phagocytes	Engorgement moderate	Moderate	±
			None	Left	384 hrs.	++	++	Slight	Edema—Mononuclear phagocytes subcapsular Eosinophiles—Subacute and chronic inflam. process about muscles	Engorgement moderate	Moderate	±
S-1 R-8	Soluble hemolytic streptotoxin. Injected into both glands	1 M.	None	Right	48 hrs.	++	+++	Slight	Edema—blood pigment changes	Marked	Marked	+++ (locally)
			None	Left	72 hrs.	0	0	No	0	0	0	0
S-1 R-5	Soluble hemolytic streptotoxin. Injected into left gland only	1 M.	None	Right	48 hrs.	±	++	Slight	Edema—wandering cells. Mononuclear cells ++. Mononuclear eosinoph. and plasma cells			+
			None	Left	96 hrs.	0	0	No	Edema—fibrosis moderate	0	0	0
S-5 R-1	Soluble and hemolytic streptotoxin	2 M.	None	Right	6 hrs.	+	++	Marked	Edema moderate. Monocytes. Eosinophiles ++	Moderate	Narrow	+++
			None	Left	72 hrs.	0	0	No	Edema. Fibrosis	0	0	0

toid culture. Eight rabbits were injected (see Chart I), six of them intra-arterially as previously outlined, and two directly into the gland. One lobe of the thyroids was removed at varying intervals from 12 hours to 336 hours in different animals and the remaining lobe was obtained at autopsy after killing the animals at intervals of 24 hours to 384 hours after the injection of the toxin. All of these animals were carefully watched, but only in two were there any apparent symptoms. In these two (Rabbits 1 and 3 in Chart I) the right eye appeared to bulge slightly and the palpebral fissure was widened. Rabbit 4 died after 70 hours of post operative bronchopneumonia. Except for hyperemia none of the glands showed any noteworthy gross enlargements. The glands were examined histologically to determine the character and degree of activity.

Because of the size of the gland it was necessary to inject small amounts. From 2 to 4 minims was the average dose used. The maximum effect was produced in 48 hours (see Fig. 1). The hypertrophy of the

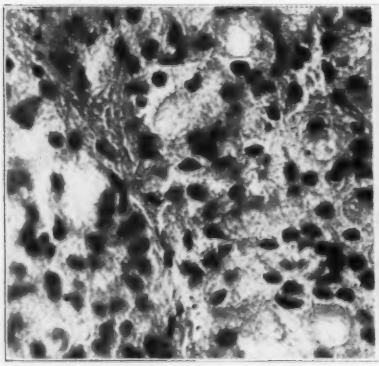

Fig. 1. Series 1, Rabbit No. 2, Right. Illustrating the type and degree of reaction following the injection of a strain of Beta hemolytic soluble streptotoxin.

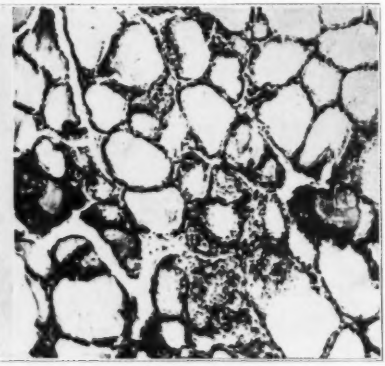

Fig. 2. (Normal) Illustrating the average microscopic appearance of the normal thyroid gland of the rabbit.

epithelial cells with the diminution of the colloid and other signs of activity is apparent when compared with the average normal (see Fig. 2). All of the glands in which there was evidence of a response to the injected material were characterized by a swelling of the epithelium somewhat comparable to a parenchymatous degeneration. In many instances there was also found an edema of the intervening supportive stroma. In some eosinophiles and plasma cells were also noted. Polymorphonuclear leucocytes were conspicuous by their absence. A minimum of hyperplasia was seen. In a few instances there were changes characterized by enlargement of the acini with blunted infoldings, duplication of epithelial layers and inclusion of epithelial cells in the colloid. The blood vessels were engorged and the lymph channels were dilated in proportion to the degree of reaction in the sinuses. When sterile distilled water, meat broth

extract, split protein products and a suspension of dried bacteria were introduced in a similar manner no noteworthy changes were observed in the thyroid glands of control animals. In one instance a sublingual abscess was produced in a rabibt without effects upon the thyroid.

Because of the difficulties in technic only a single injection was made. One must therefore estimate the results on this basis and compare them with the theoretical possibilities when toxin passes through the circulation over a longer period of time from some focus of infections from any part of the animal and particularly in the nose and throat. Infections in this location may be the disseminators of organisms to other secondary foci of infection which may serve as harbors of soluble toxin production. Such impinging toxins may act upon the thyroid directly or incite its increased action by indirect metabolism increase for the production of defensive substances.

As a further control soluble toxin from the streptococcus scarlatina (Cutter laboratory 1600 to 2300 skin test dose per cc.) was introduced into 8 rabbits, after determining its freedom from antirabbit cell agglutinins and lysins (see Chart II). Five of these were injected intravenously

EXPERIMENTS WITH DICK SOLUBLE STREPTOTOXIN
(Cutter Laboratories. 50-70,000 skin test doses per 30 cc.)
CHART II

Rabbit	Sex	Amount	Mode of Administration	Elapsed Time	Effect Upon Thyroid Gland
1	F	5 cc.	Intravenous	Found dead in 18 hours.	Hyperemia 4 plus; hypertrophied.
2	F	2.5 cc.	Intravenous	Killed after 12 hours.	Engorgement of vessels: hypertrophy of cells but little change in alveolar structure.
3	F	1 cc.	Intravenous	Killed after 12 hours.	No striking changes.
4	F	5 cc.	2.5 cc. on successive days.	Found dead day after second dose.	Capillarics engorged; no other noteworthy changes.
5	F	3 cc.	Intravenous, 1 cc. every 3 days for 3 doses.	Killed 24 hours after last dose.	Some hyperplasia of epithelium. No marked hypertrophy. Size of acini and colloid content appear within normal limits. There are some small areas suggestive of hyperplasia.
6	F	0.1 cc.	Direct into thyroid tissue.	Killed after 24 hours.	Slight focal hyperplasia in left lobe; edema is marked.
7	F	2 minims	Sup. thyroid artery.	Killed after 24 hours.	Diffuse hypertrophy and hyperplasia of epithelium. Diminution of colloid and some peripheral vacuolization.
8	F	2 minims	Sup. thyroid artery.	Killed after 24 hours.	Results of No. 7 not duplicated. Probable error.

with doses varying from 1.0 to 5.0 cc. Two of these rabbits died from toxemia within 12 hours after the injection, the lethal dose being about 2.5 cc. Two rabbits were injected intravenously on three successive days with 1.0 cc. of the scarlatina toxin, after which time they were killed. None of the thyroid glands showed noteworthy changes except for edema

and areas of focal hyperplasia. In one rabbit 0.2 cc. was injected directly into the thyroid gland. Hyperplasia and hypertrophy with edema was observed about the site of injection. In still another rabbit there was injected 0.2 cc. of the toxin into the thyroid artery. This rabbit was killed in 48 hours. A considerable degree of hypertrophy and hyperplasia was observed in the affected lobe.

CONCLUSION

(1) These experiments would seem to indicate that certain soluble streptotoxins, obtained from strains exhibiting pathogenicity for rabbits, are capable of exciting thyroid hyperplasia and hypertrophy by direct influence (injections in the gland substance and superior thyroid arteries) as well as indirectly through intravenous injections.

(2) The results obtained give further evidence of the possible rôle of the influence of soluble streptotoxins developing in some focus of infection on thyroid gland activity.

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Abstract Department

Is adrenin formed in the medulla or cortex of the adrenal gland (Le lieu de formation de l'adrénaline, substance médullaire ou substance corticale de la glande surrénale)? Abelous, J. E. and R. Argaud, Compt. rend. Soc. de biol. **103**: 129. 1930.

Beef adrenal was separated into cortex and medulla. After standing twenty-four hours, either in vacuo or on water bath, there was a decrease in the adrenin content of the medulla and an increase in that of the cortex. The adrenin was estimated colorimetrically.—J. C. D.

Unusual Addison's syndromes. Brower, A. B., Ann. Int. Med. **4**: 166. 1930.

Two cases are reported in which the syndrome was related to agenesia and hypoplasia of the adrenals in one case and to pressure atrophy of the adrenals due to polycystic kidneys in the second case.—E. L.

Extract of adrenal cortex substance. Report of its preparation and use—with some clinical notes. Coffey, W. B. and J. D. Humber, California & West Med. **33**: 640. 1930.

The authors found that suprarenal cortex when injected in graduated doses subcutaneously at definite intervals caused marked necrosis in areas of malignancy in man. This was followed by sloughing where such a process was anatomically possible, and the tumor mass usually became necrotic and liquefied. In autopsies it was noted that metastatic areas showed evidences of necrosis; this was confirmed microscopically. From a clinical standpoint patients treated with this substance were subjectively improved to the extent of feeling, sleeping, and eating better. Also, there was an increase in weight in some patients a short time after the injections. The authors deprecate the undue publicity given their work, assigning this to the eagerness of certain publications for sensational matter. They made no claim that they have a cancer cure but merely stated that they were conducting research study on malignancies of all types with a suprarenal cortex extract prepared according to their method. According to the authors, this contains an active principle which has a destructive effect upon malignant tissues without destruction of normal tissues. For reasons mentioned by the authors, it was deemed expedient to patent the process of manufacture of the extract, and now, having secured this patent, the authors freely reveal the method of its preparation. The suprarenals of sheep were found to be most advantageous. One or two injections a week are given, the dose being graduated from 1 minim and increasing to 12 minims, which is considered the maximum dose in the average patient. There are no special local irritations at the site of the injections, nor are there troublesome systemic reactions. In the past six months about 2600 patients have been under observation. Many of these have improved and returned home; 13.5% died. A majority of the patients have continued under treatment in the clinics. The authors found that x-ray and radium therapy given prior to injections delayed the action of the latter and the beneficial results expected. Other phases of this subject discussed are the carbohydrate metabolism in cancer patients, the matter of pain in malignant new growths, the question of whether improvement following suprarenal cortex injections depends upon psychic factors, and responses to questionnaires from 672 patients who had received injections for the treatment of undoubted malignancy. The authors call attention to the changes in the suprarenal cortex of individuals past the age of 40, particularly those who have died from malignancy, pointing out that the suprarenal cortices are most likely to become atrophied or deficient in their function during the cancer age of life. A brief outline of 10 case histories and a discussion of autopsy findings complete a paper on a controversial and important subject.—I. B.

The effect of adrenaline on muscle glycogen. Corkill, A. B. and H. P. Marks, J. Physiol. **70**: 67. 1920.

An effort to explain adrenaline hyperglucemia. It is pointed out from evidence in the literature that acceleration of conversion of liver glycogen into glucose does not explain this phenomenon. The experiments show that adrenaline causes a discharge of resting muscle glycogen but that this does not appear as glucose and only partly as lactic acid. Some of the carbohydrate disappearing is not accounted for, apparently being changed into some unknown product. Insulin and adrenaline together produce a similar unexplained loss, and since insulin causes increased output of adrenaline, the authors suggest that the "lost carbohydrate" following insulin administration is chargeable to this effect of adrenaline that has been discharged in response to insulin.—C. I. R.

On the nature and structure of the suprarenal cortex. McGowan, J. P., Edinburgh M. J. **37**: 545. 1930.

Chickens suffering from leucosis, in addition to myelocytic proliferation in the sinusoids of the adrenal, show dense cell masses associated with a central blood vessel. In these cell masses are groups of histioblasts surrounded by cortical cells. These cortical cells are being formed directly from the histoloblasts here in the adult organ and not as one would expect from other cortical cells.—J. C. D.

Emotional etiology of Addison's disease (Sobre la Etiologia emocional de la enfermedad de Addison). Marañon, G., Siglo med. **83**: 30. 1929.

In a number of diseases emotion may play a great part; this is especially true of certain diseases of the glands of internal secretion, assuming that emotion is necessarily a neuro-humoral phenomenon in which certain endocrines (thyroid and adrenal) collaborate. It is well known that prolonged emotions change the normal function of these organs, and contribute to the production of a pathological condition. The effects of emotional states on the thyroid are often mentioned, but not so the adrenals, despite the outstanding work of Cannon and Marañon. The author describes three cases of Addison's disease which are clearly of emotional origin. The first was described many years ago; a second was reported from the Escorial by Marañon. It is necessary to admit in explaining these cases that the suprarenal lesion existed before the psychic trauma, even though in a latent state, being clinically manifested through the influence of the emotional factor.—E. B.

The influence of epinephrine and insulin on the distribution of glycogen in rabbits. Sahyun, M. and J. M. Luck, J. Biol. Chem. **85**: 1. 1929.

Rabbits fasting for 24 hours were killed by stunning. The liver and sartorius muscle were speedily excised and their glycogen content determined by Pflüger's method. Liver glycogen fell after subcutaneous injection of 1 mgm. of epinephrine, reaching its lowest value after 1½ hours. Three hours after injection it had risen beyond the original value and continued to rise up to the 18th hour, when it amounted to an average of 3.7 against the original 0.5%; thereafter it fell rapidly to the fasting level. Muscle glycogen decreased markedly; the fall occurred in the first hour after injection and practically the same level was maintained for the next 42 hours. Administration of insulin along with epinephrine inhibited the initial fall in liver glycogen. Epinephrine is regarded as a promotor of glycogenolysis in liver and muscle and inhibitor of peripheral utilization of blood sugar, whereas insulin, on the contrary, is pictured as inhibitor of hepatic glycogenolysis and promotor of utilization of blood sugar in the muscle.—G. T. Cori.

Diagnosis of tumor of the carotid body. Bodine, M. W., Pennsylvania M. J. **34**: 17. 1930.

This is a brief review of the subject. The author points out that the differential diagnosis of carotid tumor is difficult. The diagnosis is based on the presence of a smooth, ovoid, firm, slowly-growing, single tumor in the neck, which catches, fixes, and carries with it the carotid artery. Pain and tenderness are inconstant. It may or may not produce pressure symptoms

such as result from involvement of recurrent laryngeal, hypoglossal, pneumogastric, facial, phrenic, or sympathetic nerves. It may or may not vary in size when pressure is applied over the common carotid artery. Bruit and thrill are inconstant.—I. B.

Histological details of the reaction of the thyroid to extracts of the anterior lobe of the hypophysis (Particularites histologiques de la réaction de la thyroïde aux extraits de lobe antérieur d'hypophyse).

Changes produced on the function of the thyroid by injections of hypophysis and their morphological indications (Indications apportées par la méthode des injections hypophysaires sur le fonctionnement de la thyroïde et ses tests morphologiques). Aron, M., Compt. rend. Soc. de biol. **103:** 145. 1930. Id. 148.

Dogs, cats, guinea pigs, and rabbits were used. The hypophyseal injections produced over-activity in the thyroid, as shown by the condition of the individual cells and of the follicles. The histology of the follicular cycle, growth, accumulation of colloid, and repair is given and the changes indicative of over-activity described.—J. C. D.

The impermeability of the placenta to the substance from the anterior lobe of the pituitary which acts on the thyroid (Sur l'imperméabilité du placenta a la substance préhypophysaire active sur la glande thyroïde). Aron, M., Compt. rend. Soc. de biol. **103:** 151. 1930.

In guinea pigs the thyroid shows signs of great activity within two days of pituitary injections. Such injections into pregnant animals failed to produce any reaction in the thyroids of their fetuses. This substance, therefore, does not penetrate the placenta from mother to fetus.—J. C. D.

Serum calcium in guinea pigs. Influence of hyperthyroidism and castration (Sérocalcémie chez le Cobaye. Influence de l'hyperthyroïdie et de la castration). Blinoff, A., Compt. rend. Soc. de biol. **103:** 187. 1930.

There were slight changes. The thyroid fed animals showed an increase, the castrated a decrease, and the hyperthyroid-castrates the highest calcium content.—J. C. D.

Endocrinopathies: The thyro-pituitary syndromes. Diagnosis and treatment. Eidelsberg, J., M. Clin. North America, **14:** 425. 1930.

This is a general consideration of the subject with three illustrative case histories. The author emphasizes the relationship which exists between the various ductless glands, particularly between the thyroid and pituitary, so often pronounced in definite clinical syndromes in children. Treatment should take into account the need for administration of more than one gland, depending upon clinical manifestations. X-ray treatment of the thymus may be required in these patients. Despite the fact that many cases of pituitary involvement concern bilobar pituitary deficiency, one lobe may be affected and treatment should be instituted accordingly. With respect to thyroid opotherapy maximum doses should be given. The author has seen patients requiring as high as 15 grains of thyroid gland a day, there being little response with smaller doses. The administration of pituitary substance may be either hypodermically or by mouth. The author advises anterior lobe substance to be given in doses as high as 15 to 45 grains by mouth. In pituitary opotherapy the best results are obtained by a combination of subcutaneous and oral administration. The prognosis depends largely upon how early the patient is placed under appropriate attention. In early cases results are highly satisfactory. Late cases, while not altogether hopeless, cannot be expected to achieve marked benefit from treatment.—I. B.

Contribution to the etiology of obesity (Zur Frage der Aetiologie der Fettsucht). Kup, J., Endokrinologie, **6:** 102. 1930.

From a rather great amount of autopsy material, cases of obesity were collected and all of the endocrine glands examined from the pathological, anatomical and histological points of view, in order to get particular information

about the etiology of obesity. There were 31 cases, 29 females and two males. The cases were separated into several groups according to the assumed etiology: (1) Obesity after castration, 3 cases. No typical transformations of the pituitary body; hypertrophy of the islets in the pancreas. (2) Pituitary obesity, 2 cases. Transformations of the pituitary body. (3) Pineal obesity, 2 cases. With transformations in Marburg's sense. (4) Pineal obesity, 2 cases. (5) Pancreatogenous obesity, 14 cases. (6) Thyrogenous obesity: No self-observed case of purely thyrogenous obesity. Transformation of the thyroid gland in two cases which have been mentioned with other groups. (7) Pluriglandular obesity, 1 case, with which transformations of various endocrine glands were found simultaneously; transformations which themselves might have caused obesity. (8) Cerebral Obesity: No personal case for which this name might safely be applied. The paper discusses the assumption that diseases in the region of the hypothalamus cause obesity. (9) Obesity and infectious diseases: The interpretation of Carnot and of the well known clinical phenomena are discussed. (10) Diabetes and obesity, 1 case. (11) Other causes of obesity. One case of obesity caused by inactivity of muscles with paraplegia. In five cases no relation between the condition of endocrine glands and obesity was found. Further explanations are expected in regard to these cases, as well as to cases of obesity after infectious diseases and cases of heredofamilial obesity from more extensive investigation of endocrine glands.

—H. M. Bosshard.

Recent endocrinology. Lisser, H., California & West. Med. **33**: 545. 1930.

This is an interesting review on recent developments in the field of endocrinology, including the relationship between diabetes and arteriosclerosis, the subject of pituitary diabetes, pituitary infantilism with hypoglycemia, acromegaly and amenorrhea, anterior pituitary hormone diagnosis of pregnancy, antagonism of anterior pituitary growth and sex hormones, the hormones of the posterior pituitary, the matter of parathormone, and that of hyperparathyroidism. The author concludes this interesting paper with comments on the present status of organotherapy.—I. B.

Endocrine correlations. The embryohormonic relations of the suprarenal cortex mesothelial tissue. Moehlig, R. C., J. Lab. & Clin. Med. **16**: 118. 1930.

That embryology supplies the solution to many of the correlations between endocrine glands has been the author's contention for many years. This paper is a brief excellent analysis of ductless gland interrelationships, dealing particularly with the embryohormonic relations of the suprarenal cortex to mesothelial tissues. The author defines embryohormonic control as the selective action of endocrine secretions on tissues depending upon their embryologic origin.—I. B.

The influence of hormones on sugar distribution and cell permeability (Der Einfluss von Hormonen auf Zuckerverteilung und Zellpermeabilität im tierischen Organismus). Siegel R., Klin. Wchnschr. **8**: 1655. 1929.

The degree of permeability of the tissues for sugar has been studied under various conditions by means of determining the free sugar content in the blood on the one hand and different organs (liver, muscles, etc.) on the other hand. In normal fasting mice the blood sugar is about twice as high as in the brain and 1½ times as high as in the muscles. Under the influence of insulin sugar enters the muscles in an increased amount and the sugar content of the tissues and of the blood become about equal, provided that small "physiological" doses have been given. After the extirpation of the pancreas, on the contrary, the tissue sugar does not rise in accordance with the blood sugar. Thyroxin does not influence the permeability for sugar of the cell membranes. The influence of the above mentioned hormones upon the liver is different. Insulin inhibits the diastatic splitting of glycogen in the liver tissue, while this process is increased in the livers of pancreatectomized animals. Thyroxin increases the diastatic effect; thyroidectomy diminishes it. Thyroxin is therefore to be considered as a direct antagonist of insulin in this particular respect. Adrenalin exerts two different effects upon the liver. It inhibits the diastatic glycogenolysis inside the liver cell directly, but this effect is mostly overcome by the usual experimental doses through the stimulating effect on the sympathetic system, which on the contrary increases glycogenolysis. The author

assumes that under physiological conditions only the first named effect which is similar to that of insulin takes place. In the peripheral tissues adrenalin inhibits the entrance of blood sugar into the cells.—W. Raab.

A substance that stimulates the central nervous system and its functions. Steinach, E. and H. Kun, Med. Klin. **25**: 1273. 1929. Abst., Arch. Neurol. & Psychiat. **23**: 1274.

Watery extracts of the central nervous system are shown to contain a substance capable of stimulating reflex activity. The extract was injected into frogs, and numerous indifferent substances were used in control animals. Over 800 animals were tested by means of graduated chemical stimuli, and an increase was observed of from 400 to 600% in reflex activity as compared with control animals. Attempts were also made to estimate more general performances by the experimental and control animals by observing their ability to catch flies in a closed chamber, with the result that in most cases the former showed the greater activity. The active substance, to which the author gives the name "centronervin," is not limited to any one portion of the nervous system or to any one species of animals; the same results were obtained with material prepared from the gray matter, white matter and spinal cords of frogs, rats, dogs and human beings. It is thermolabile and probably hormonal in nature. The author supposes that it guides the activity of the central nervous system, and he raises the question whether mental retardation, psychic anomalies and some disease of the nervous system may not be due to insufficient or abnormal formation of this substance.

Changes in the blood serum following combined castration and hyperthyroidism (Modifications humorales dans le syndrome athyro-anorchitique expérimental). Werner, G., Compt. rend. Soc. de biol. **103**: 194. 1930.

Rabbits and sheep were used. Blood sugar and calcium were reduced, urea nitrogen and cholesterin were increased, while the chlorides and the alkaline reserve were unchanged.—J. C. D.

Recently discovered endocrine diseases. Hyperepinephrinism, hyperinsulinism, and hyperparathyroidism. Wilder, R. M., Internat. Clin. **1** (Series 40): 293. 1930.

This is a brief discussion of the more recent endocrine entities. Adrenal medullary tumor is mentioned with relation to paroxysmal hypertension. The more significant observations concern the association of chromaffin tumors with paroxysmal hypertension in which the blood pressure during the attacks may rise to 280/110. Illustrative cases are those of C. H. Mayo, Pincoffs, and Shipley. The clinical picture seems to duplicate that resulting from an injection of epinephrin in susceptible individuals. The removal of the tumor in 2 cases was followed by apparent recovery. The author therefore concludes that certain forms of hypertension may be due to excessive epinephrin secretion. The association of a tumor of the islands of Langerhans with the syndrome of hyperinsulinism is a matter of increasing importance. It is observed that reduction of the mass of the pancreas in cases without tumor or resection of the tumor when one existed suppressed such attacks. Also measures provoking hypertrophy of the islands of Langerhans in dogs produce a condition analogous to that observed in patients suffering with the symptoms of hyperinsulinism. Illustrative case histories of hyperinsulinism are cited in which the major clinical manifestation is that due to hypoglycemia. Cases of hyperparathyroidism are becoming increasingly frequent. The diagnosis depends upon the presence of a high blood-calcium and a low blood-phosphorus in cases of skeletal disease with hypertrophy or actual tumor of the parathyroid glands, and the control of these abnormalities by the surgical removal of such tumors. Occasionally there may exist hypersecretion of morphologically normal glands. The author suggests that the importance of the three endocrine disturbances described is enhanced by the fact that treatment is satisfactory in each. Where tumors were responsible the direct surgical attack has been highly successful. Where overfunction has been due to overactivity of otherwise normal glands, the results of operation have been less uniform. Further investigation of the possible usefulness here of Roentgen therapy is desirable. In cases of osteitis and osteomalacia from hyperfunction of the parathyroids, benefit may be derived from large doses of cod-liver oil and ultra-violet light.—I. B.

The relation of the lipids to physiological activity. I. The changes in the lipid content of the corpus luteum of the sow. Bloor, W. R., Ruth Okey and G. W. Corner, J. Biol. Chem. **86**: 291. 1930.

The ovaries of 21 non-pregnant sows were chosen to cover as completely as possible the different days of the cycle. Eight samples taken at different stages of pregnancy were also examined. Dating was based upon histological examination of fixed and stained sections of ovary and endometrium. Chemical examination of tissue samples included determination of lecithin and fat by the oxidative procedure of Bloor and of cholesterol and cholesterol esters by the digitonin precipitation procedure of Okey. During the period of growth lecithin content increases, and lecithin ester cholesterol ratios are very high. Ester cholesterol increases and lecithin decreases sharply as degeneration proceeds. The data are discussed in support of the hypothesis that the phospholipid content of a tissue is a function of its physiological activity. The relation of free cholesterol to activity is less clear, but appears to be similar to phospholipid, while cholesterol esters seem to be related to inactivity or retrogression.—R. Okey.

Contribution to the study of macrogenitosoma with a description of a case with the macrogenital syndrome accompanied by pubertal intersexuality of the gynecomastic type (Contribucion al estudio de la macrogenitosomia con descripcion de un case de sindrome macrogenitosomico e intersexualismo puberal de tipo ginecomastico). Bonilla, E., Arch. españ. de pediat. **12**: 591. 1928.

The author describes the different clinical forms of macrogenitosoma precox according to the origin of the disturbance by the genital glands, suprarenal or epiphysis. He refers to a typical case of purely genital origin in a boy of twelve years. The major interest lies in the association of genital hyperfunction with intersexual characters, so called, such as gynecomastia; and feminine hair distribution rather than masculine hair distribution. In the literature there are similar observations. To explain this paradoxical association the author assumes a bi-sexual mechanism in accordance with the concepts of Marañon concerning two puberty factors—one for the feminine sexual condition and the other for the masculine, but both of which are present in the single individual.—G. M.

The physiological activity of the crystalline female sex hormone in the Allen-Doisy test (Uber die physiologische Wirksamkeit des krystallisierten weiblichen Sexualhormons im Allen-Doisy Test). Butenand, A. and E. von Ziegner, Ztschr. f. physiol. Chem. **188**: 1. 1930.

The authors have obtained a crystalline preparation of the female sex hormone, progynon, by fractional crystallization and sublimation. The pure and concentrated preparation is believed to have a potency of as much as 35 or 40 million mouse units per gram. This potency is higher than that obtained in any other previous substance. The crystalline preparation, progynon, has a constant melting point of 243-245 degrees. Its potency remains unchanged after repeated purification processes and sublimation.—L. C. Wyman.

Insulin and onset of oestrual cycles (Influencia de la insulina sobre la iniciación de los ciclos estruales en la rata blanca normal). del Castillo, E. B. and C. Cardini, Rev. Soc. argent. de biol. **6**: 225. 1930.

Daily injection of 0.1 unit of insulin in rats (20-23 days old) did not modify time of opening of vagina, appearance of first oestrus and rhythm of cycle as compared with control litter mates.—J. T. Lewis.

Sex hormones in the blood serum of mares. II. The sera of mares from the 222nd day of pregnancy to the first heat period post-partum. Cole, H. H. and G. H. Hart, Am. J. Physiol. **94**: 597. 1930.

Blood serum was obtained from mares late in pregnancy and injected into immature rats. It produced reactions in the genital system of the rats very dissimilar to those obtained from serum collected during the early stages of pregnancy. From about the middle to approximately the end of the gestation period, a dose of 25 cc. or more regularly produced a positive vaginal reaction as characterized by a smear of oestrum, a slight enlargement of the uterus,

and an inhibition of the development of the ovaries. This would indicate the absence of ovary stimulating factors in recognizable amounts in the serum during late stages of pregnancy. Oestrin and possibly other ovarian factors were no doubt responsible for the reactions produced. The response described above was occasionally absent in serum as early as a week before the end of pregnancy and was invariably absent in the post-partum period.—H. H. Cole.

Further observations on an ovary-stimulating hormone of the placenta. Collip, J. B., Canad. M. A. J. **22**: 761. 1930.

Collip gives an exhaustive review of the subject and points out that Hirose in 1920 associated injection of placental suspensions with effects on the ovary before attention was directed to the effect of anterior pituitary. Several methods are given in detail for preparation of human placental extracts for clinical use, and in a state of crystalline purity. The vaginal smear method has been employed for routine testing of the extracts. The difficulties of quantitative standardization are discussed. Corpora lutea have seldom been found in ovaries of immature rats treated with the pure product, supporting Wiesner's hypothesis that there is a separate principle which stimulates luteinization. Evidence is given for and against the theory that the placenta is the ductless gland of pregnancy. Extracts of anterior pituitary, prepared in corresponding fashion to those of placenta, are non-oestrogenic. The placental hormone is effective orally, and does not appear to be decomposed by pepsin or trypsin. Prolonged treatment of normal adult rats seems to have no effect upon the cycles nor upon impregnation nor lactation and does not produce marked hypertrophy of the ovaries nor superfetation. Massive doses produce little or no interference with normal sex functions in normal individuals. Abortion has not been produced. No significant change is produced in the blood chemistry (rabbits and dogs). In normal female dogs and rabbits so treated there is no sign of luteinization of the ovaries. Immature rats brought to premature maturity by treatment commence to manifest the cyclic changes at once. The earliest instance of impregnation observed has been in the third cycle; absence of impregnation during the first cycle may be associated with absence of the luteal phase. After two weeks of daily injections with known potent extracts proved free from oestrin, H. B. Van Dyke obtained oestrus in 5 completely hypophysectomized rats, and this may be viewed as evidence against the placental origin of the hormone. The name emmenin is suggested. An active preparation precipitated from aqueous solutions by alcohol between the ranges of 65% and 85% is apparently of protein nature and seems to contain another hormone. It stimulates the female to hypertrophy of the ovary due chiefly to formation of corpora lutea. It is ineffective orally, and its properties are similar to those of the so-called anterior pituitary principle of pregnancy urine.—A. T. C.

The preparation of the crystalline ovarian hormone from the urine of pregnant women. Doisy, E. A., C. D. Veler and S. Thayer, J. Biol. Chem. **86**: 499. 1930.

An extraction procedure based upon the well known solubility of the hormone in oils has been used. Though the procedure is more time-consuming than the use of the continuous liquid extractor after the apparatus of the latter is set up and operating, it is believed that a number of workers may be glad to obtain a supply of hormone with so little expenditure of labor as is involved in stirring a sample of acidified urine with olive oil. From a liter of urine we obtained 700 rat units of hormone by agitating with a motor-driven stirrer with 50 cc. of olive oil for 20 or 30 minutes. A method of purification is described in which a crystalline product is obtained. Upon repeated recrystallization the crystals were found to be exceedingly active physiologically. The potency of the crystals were found to exceed 3000 rat units per mgm. The crystals occur in two forms depending on the nature of the solvent. The physiological assay showed the same potency. It is possible to convert one form into the other. The evidence in favor of the view that the crystals were in reality the hormone was stated as follows: (1) The potency of the crystals exceeded the potency of any non-crystalline product that had been studied by ourselves or other investigators. (2) Several preparations of the platelet crystals had the same potency. (3) Careful microscopic examination revealed only one kind of crystals. (4) Repeated recrystallization did not diminish the potency and the mother liquors tested were less active than the crystals.
—S. A. Thayer.

The endocrines in gynecology with special reference to dysmenorrhea and other menstrual disorders. Gabrielianz, A. G., Illinois M. J. **58**, 193. 1930.

Folliculin is a powerful remedy in cases of hypofunction of the ovary. At present the action of folliculin obtained from animals is more satisfactory than that obtained from human sources. Sistomensin is a powerful hemostatic in uterine hemorrhage of endocrine origin. Whole ovarian gland is less satisfactory in relieving dysmenorrhea than is pluriglandular therapy. After a course of folliculin, it is advisable to continue treatment by agomensin (lipamin). In the treatment of amenorrhea of ovarian origin, folliculin treatment is more powerful than pluriglandular therapy. Pluriglandular therapy is more satisfactory than agomensin (lipamin) alone. The author quotes Eidelsberg to the effect that persistency and patience are required in the treatment of endocrine disturbances.—I. B.

On the sex hormones, especially feminin (Über Sexualhormone insbesondere das Feminin). Glimm, E. and F. Wadehn, Biochem, Ztschr. **219**: 155. 1930.

The female hormone occurs in an ether soluble and an ether insoluble form. The ether insoluble form as it occurs in urine is not in the form of an alkali salt. It is designated V-feminin. It makes up a considerable portion of the female hormone content of urine. Urine from children contains between 100 and 200 mouse units per liter. This rises with the increasing age of the individual and at puberty may be as high as 1000 mouse units per liter. This is true of both male and female urine. The female hormone content of male urine cannot be accounted for entirely by the occurrence of this hormone in foods. Chloroform extracts of male urine injected into young female mice produce oestrus while in the male they have an inhibiting effect.

—R. G. Gustavson.

Relation of the follicular and corpus luteum hormones in the production of progestational proliferation of the rabbit's uterus. Hisaw, F. L. and S. L. Leonard, Am. J. Physiol. **92**: 574. 1930.

A corpus luteum hormone from sows' corpora lutea, prepared essentially by acid alcoholic extracts, is able to produce several reactions usually attributed to the corpus luteum, among which are the histological changes in the uterus typical of pseudopregnant rabbits, sometimes known as progestational proliferation. The corpus luteum hormone can produce these changes only while the uterus is under or recovering from the influence of oestrin. Rabbits castrated in heat and injected with the corpus luteum hormone will show the typical histological changes characteristic of pseudopregnancy in five days. If the uterus of the rabbit is in the castrate condition, corpus luteum hormone alone cannot elicit the progestational changes. If such castrate rabbits are given follicular hormone until they are in heat and then given doses of corpus luteum hormone, the reaction can be obtained. The corpus luteum hormone alone is not able to maintain the progestational condition in an animal castrated in oestrum as long as it normally exists during pseudopregnancy. It is suggested that follicular hormone in definite amounts is needed along with the corpus luteum hormone. The follicular hormone is growth-promoting while the corpus luteum hormone acts as a modifier of structures already put down by oestrin. It is also possible to override the effects of the corpus luteum injections by large doses of the follicular hormone. Therefore, there exists a definite quantitative relationship between the follicular and corpus luteum hormones in producing and maintaining progestational changes in the uterus of rabbits. This relationship is upheld in all corpus luteum reactions so far studied in this laboratory.—S. L. Leonard.

Experimental cryptorchidism of pigs. Korenchevsky, V., J. Path. & Bact. **33**: 683. 1930.

Two domestic pigs were made cryptorchid and compared with two castrated litter mates. The final weight of the cryptorchid pigs was greater than that of the castrated pigs by 13.2%. The amount of retroperitoneal fat, but not that of subcutaneous fat, was slightly increased in cryptorchid pigs. The cryptorchid pigs looked to be more hairy than the castrates. Histologically,

in the cryptorchid testes the seminiferous tissue seemed to have disappeared, the Sertoli's cells were normal, while the hyperplasia of the Leydig's cells was greater than in any other species of animal made cryptorchid.—Author's Abst.

The influence of cryptorchidism and of castration on body-weight, fat deposition, the sexual and endocrine organs of male rats. Korenchevsky, V., J. Path. & Bact. **33**: 607. 1930.

Experiments were performed on 148 rats, belonging to 34 litters. Each litter was divided into a control group and one or two groups which were made cryptorchid (one sided or both sided) or castrated. The results obtained were compared with those on 37 other rats, divided into similar groups, but belonging to different litters. Experimental both-sided cryptorchidism did not influence appreciably, the body-weight of rats nor the weight of the hypophysis. In most rats there was an increased deposition of body-fat, a considerable atrophy of the penis, prostate, seminal vesicles and thyroids, and a slight and less constant atrophy of the adrenals. These changes reached their maximum in about two or two and a half months. The rats killed about six months after the operation did not display a greater degree of the changes. If only one testis was made cryptorchid and the other left normal the changes were less constant and usually much less pronounced. No atrophy of the prostate and seminal vesicles was observed. Cryptorchidism of one testis did not influence the weight of the second testis, left normally situated. Castration of male rats was followed by a decrease in body-weight, an increase in fat deposition, a striking atrophy of the penis, prostate and seminal vesicles and a considerable atrophy of the thyroids. The adrenals and the hypophysis were hypertrophied. The changes obtained after cryptorchidism, when compared with those after castration, may be divided into four groups according to their consistency and degree. (a) Decrease of the body-weight and hypertrophy of the adrenals and of the hypophysis are present in castrated rats and absent in cryptorchid rats. (b) Atrophy of the sexual organs is a constant, and the most highly developed change in castrated animals, while in cryptorchids it is much less pronounced and may even, sometimes, be absent. (c) Obesity varies in degree and constancy both in castrated and cryptorchid animals, being on the average more pronounced in castrated rats. (d) Atrophy of the thyroids develops to the same degree and as consistently in both cryptorchid and castrated animals. The analysis of the above data shows the presence of sexual hormones both in the seminiferous and in Leydig's testicular cells and makes most probable that: (a) The seminiferous cells secrete hormones which stimulate metabolism and the thyroids. (b) Leydig's cells produce hormones which are necessary for the normal growth and development of the adrenals and the hypophysis. (c) Hormones stimulating the sexual organs are produced by both the seminiferous and the Leydig's cells. (d) The development of the sexual organs is influenced by the testicular hormones in correlation with (or through) the endocrine glands, namely, the hormones of the Leydig's cells with the hypophysis and the adrenals, the hormones of the seminiferous cells with the thyroids. Obesity develops after cryptorchidism and castration as the result of a decrease (in cryptorchids) or exclusion (in castrates) of the hormones which are produced by the seminiferous cells and which stimulate metabolism both directly and by stimulation of the thyroids. The differing degree or even absence of obesity in some cryptorchid and castrated animals is explained by (a) the varying degree of atrophy of the seminiferous cells (in cryptorhids) and of the thyroids (in both cryptorchid and castrated animals); (b) the varying degree of change after castration in the hypophysis, adrenals and the islets of Langerhans; (c) the removal of the influence of hormones depressing metabolism which are also present in the seminiferous cells, the proportionate relationship of which to the hormones stimulating metabolism, varies in different animals. A comparison of the weight of the fat and of the organs investigated, in rats belonging to the same litter, does not usually give large variations in the figures obtained, whilst in rats belonging to different litters the variations may be very great. The variations in the weights of fat, sexual organs and thyroids are increased if the rats compared not only belong to different litters, but are of different ages. Therefore, incorrect conclusions may follow the use of control rats which belong to different litters from those rats on which the experiments were performed. When, however, a large number of animals is used for comparison, the averages obtained may approximate fairly closely to each other, provided the rats were bred and fed under the same conditions.—Author's Abst.

Experimental prolongation of pregnancy by means of corpus luteum extracts (Experimentelle verlängerung der Schwangerschaft mittels corpus luteum-extrakten; vorläufige Mitteilung). Miklós, L., Zentralbl. f. Gynäk. **54**. 1755. 1930.

In sixteen rats injected daily, from the 16th and 17th days of pregnancy, with an alcohol ether soluble lipoid in olive oil, onset of labor occurred from the 23rd to 28th day. In twelve controls it occurred from the 19th to 24th day. According to results in this small series corpus luteum extract prolongs pregnancy. Complete details of the preparation of the extracts are not given.
—J. P. P.

Seminal-vesicle and prostate function as a testis-hormone indicator; the electric ejaculation test. Moore, C. R. and T. E. Gallagher, Am. J. Anat. **45**: 39. 1930.

An ejaculation can be produced at will in the guinea pig by stimulation on the head with an electric current and these stimulations can be repeated at intervals for an unlimited period of time. Ordinarily one stimulation and ejaculation per week was employed. The discharge consisted of secretions from the prostate gland and seminal vesicles with spermatozoa from the epididymis. Castration causes a loss of function of these accessory reproductive glands, hence castrated males fail to give an ejaculation upon proper stimulation. Males with testes in the abdomen (experimental cryptorchidism) give normal ejaculations, hence the phenomenon is under the control of the internal secretions of the testes and it has been utilized as a means of detecting the active principle of the testis hormone when injected into castrated males. Employing the electric ejaculation test as a means of detecting active preparations containing the testis hormone after subcutaneous injection it was possible to prevent typical castration changes from developing in animals after operation and to restore function to these accessory glands within a period of two weeks, despite the fact they had been in a functionless state for many months, due to castration. The source of the hormone was lipoid extracts of bull testes and injections must be done daily since there is no hormone storage within the body.—Authors' Abstract.

Rat prostate cytology as a testis hormone indicator and the prevention of castration changes by testis extract injections. Moore, C. R., Dorothy Price and T. F. Gallagher, Am. J. Anat. **45**: 71. 1930.

Castration in the rat produces marked changes in the three lobes of the prostate gland. These involve acinus shrinkage, increase in connective tissue stroma, reduction in secretory epithelial height, loss of specific light areas in secretory cells (of middle and posterior lobes) and marked reduction and fragmentation of the Golgi apparatus. Mitochondria were not markedly changed by castration. Castration changes are apparent in cytological preparations from animals castrated for five days. The cytological changes of castration were utilized to determine the presence or absence of the internal secretion of the testis in bull testis extracts administered subcutaneously. By means of testis extracts it was possible to: (a) prevent the development of castration changes for indefinite periods; (b) rebuild degenerated prostates of animals castrated for from thirteen to one hundred days up to the normal condition within twenty days; and (c) to develop the arrested prostate in animals prepubertally castrated. The testis extracts could entirely substitute for the normal testis in controlling the structure and function of the prostate gland. Continuous injections are required to maintain the normal state of the gland for a lapse of two days in injections were detectable in cytological preparations of this organ.—Authors' Abstract.

Rat seminal vesicle cytology as a testis hormone indicator and the prevention of castration changes by testis extract injections. Moore, C. R., Winifred Hughes and T. F. Gallagher, Am. J. Anat. **45**: 109. 1930.

The seminal vesicles of the rat show definite cytological changes within two days after castration and progressively more marked changes up to about twenty days when the full effects of castration are evident. The changes involve loss of secretion granules from secretory cells, shrinkage of acini, involution of the secretory epithelium, diminution and fragmentation of Golgi

bodies. Castration can be diagnosed within two days after testis removal. These changes have been utilized to determine the hormone state of the organism and to study the effects of injecting extracts of bull testes into castrated animals. Daily subcutaneous injections of testis extract into castrated males for twenty days after operation have given a range of effectiveness of the extract proportional to the amount of lipoid material injected. Castrated males have been maintained in a normal condition for indefinite periods of time. Animals castrated for ten days and showing the definite cytological changes in the vesicles have been returned to normal conditions by a subsequent ten days of daily injections. Adult animals castrated for months have been returned to normal conditions within a period of thirty days. Prepubertally castrated animals of several months standing have been restored to normal states within thirty to forty days by injecting the testis extracts. The testis extracts containing the testis hormone were prepared from the lipoid fraction of bull testes.—Authors' Abstract.

The variations in the lipids of the uterine mucosa in the pig. Okey, Ruth, W. R. Bloor and G. W. Corner, J. Biol. Chem. **86**: 307. 1930.

The lecithin, cholesterol, cholesterol ester and fat content of 16 samples of endometrium dated as in the previous study are reported. The water content of this tissue is variable and high. There is a definite but not large increase in the percentage of lecithin at the time of greatest elaboration of the endometrium. This is, however, accompanied by an increase in free cholesterol. Hence the lecithin-cholesterol ratios are not so high as those in the corpus luteum at the same time. The amount of cholesterol ester in the endometrium is always low and there is never any evidence of accumulation of this substance in endometrial tissue. The data on lipid content of endometrium and corpus luteum are discussed in relation to the specific physiology of the sexual cycle. —R. Okey.

On the synergism between oestrin and oxytocin. Parkes, A. S., J. Physiol. **69**: 463. 1930.

Oxytocin is practically ineffective in producing abortion in mice but pituitrin is effective due to the presence of vasopressin. Oestrin was effective in divided doses of amounts of 380 units. When divided doses of smaller amounts were followed by injection of 1 unit of oxytocin, immediate abortion resulted. This result is believed to be due to oestrin sensitization of the uterus to oxytocin.—C. I. R.

The inhibition of oestrin by corpus luteum extracts. Patel, J. S., Quart. J. Exper. Physiol. **20**: 245. 1930.

An effort was made to test the validity of the theory that the action of the corpus luteum is to inhibit the occurrance of the first phase of ovarian activity. The method of preparing corpus luteum extracts is described. In mice the extract (Kythin) produces inhibition that can be maintained for as long as 40 days without ovarian injury. The oestrogenic stimulus is reduced so that oestrin secretion is inhibited.—C. I. R.

The practicability of the Aschheim-Zondek test for pregnancy. Robertson, E. M., Edinburgh M. J. **27**: 124. 1930. (Transactions of Edinburgh Obstetrical Soc.)

Excellent plates show the gross and microscopic appearance of the ovaries in mice injected with urine from pregnant women. In 479 controlled cases of pregnancy, the test showed an accuracy of over 98%. It was positive in cases of hydatidiform mole and of chorion epithelioma.—J. C. D.

A case of genital deformity (Un caso de dismorfismo genital). Soler y Dopff, J., F. Forcada Gelaver and C. Soler y Dopff, Archives de Medicina Cirugia y Especialidades, **28**: 714. 1928.

The case of a twenty-one year old girl with a double inguinal hernia is described. She had never had a menstrual period. Operation on the right hernia revealed a glandular structure in the hernia pouch about the size of a dove's egg, which suggested a "hernia ovary." In the left sac a similar but larger structure was found. Histological examination showed this to be a

testicle in full activity. This description does not coincide with most descriptions of androgynus individuals, in which changes of secondary sex characters arise from atrophy or hypo-function of the recessive gland, but is directly the opposite. The fact that the glandular body in the right hernia sac was not studied removes all its value, since the solution of the problem was surely there, as that gland was probably a more or less degenerate ovary. To suppose, as some authors do, that a typical feminine form and maternal instincts can co-exist along with masculine erotic tendencies in a male gonad is inadmissable.—E. B.

Clinical value of the Aschheim-Zondek test for pregnancy. Stone, B., South. M. J. **23**: 747. 1930.

This method depends on the presence of anterior pituitary hormone in the urine of pregnant women in large enough quantities to cause characteristic ovarian changes when injected into young mice. The original authors claim a 98% accuracy for the test. Dr. Stone found it accurate in over 90%. The paper includes a description of the test and the possible causes of inaccuracy.
—J. C. D.

On the endocrine basis of mating the mouse. Wiesner, B. P. and L. Mirskaia, Quart. J. Exper. Physiol. **20**: 273. 1930.

Mating was induced in ovariotomised mice by injections of follicular juice and cornifying extracts from pregnancy urine. The authors suggest discarding the terms "folliculin" and "oestrin" in favor of "Allen-Doisy factor" or "Alpha hormone."—C. I. R.

The unitary versus the multiple hormone theory of posterior pituitary principles. Abel, J. J., J. Pharmacol. and Exper. Therap. **40**: 139. 1930. Id. 170.

The author contends that posterior pituitary extracts contain only one hormone which retains all the known physiological properties demonstrated, in the same relative proportions but with greater potency than the standard but from which it is possible, by hydrolysis, to prepare products displaying individual physiological effects, but with a higher assay value. The evidence and arguments are too voluminous to abstract.—C. I. R.

The relation between anterior pituitary hormone (Prolan) and the male generative glands (Ueber Beziehungen Zwischen Hypophysenvorderlappenhormon). Borst, M., Deutsche med. Wchnschr. **56**: 1117. 1930.

The effects of anterior pituitary hormon (prolan) on male mice were studied on three distinct age groups with different dosage and varied duration of treatment. In the youngest group, animals up to 26 days old, the germinative portion of the testis appeared specially sensitive, showing irritation and injury. The other 2 groups, one maturing (26-40 days old), the other fully matured sexually (40 days or more of age) showed a greater resistance of the germinative tissue but a gradually varying reaction of the interstitial substance.—E. P. D.

The selective vasoconstrictor action of pituitary pressor extract. Clark, G. A., J. Physiol. **70**: 53. 1930.

By means of the plethysmograph the distribution of blood after injection of pitressin in vagotomized cats under chlorolase, amytal or urethane anaesthesia, or after decerebration was studied. The intestinal supply of blood is diminished, that of the muscles increased due to vasoconstriction in the former, plus occasional improved cardiac activity. The skin and connective tissues contain less blood except when the blood pressure is increased.—C. I. R.

On the existence of a fourth hormone, thyreotropic in nature, of the anterior pituitary. Crew, F. A. E. and B. P. Wiesner, Brit. M. J. **1**: 177. 1930.

In this preliminary paper the authors show that the extracts of the anterior lobe which induce metamorphosis in axolotls need not contain either the gonadotropic factors (Rhofactors) which stimulate and determine the

function of the ovary and testicle, nor the growth factor. On the other hand, even such extracts which contain the gonadotropic hormone in high concentration do not stimulate the thyroid nor do they induce metamorphosis. The thyroid stimulating factor, the existence of which was demonstrated by Uhlenhuth and associates is therefore not identical with any of the other anterior lobe hormones so far described. The provisional term "thyreotropin" or the less committing "theta-factor" is proposed.—B. P. Wiesner.

The use of thymophysin in labor. Davis, M., New England J. Med. **203:** 771. 1930.

Thymophysin is a combination of pituitary with thymus extract devised by Dr. Nicholas Temesvary of Budapest. The author reports its use in fifty carefully studied cases. He concludes: 1. Thymophysin can be given as soon as regular labor is established. 2. There is apparently no danger of tetanic contraction of the uterus. 3. Thymophysin definitely shortens labor. 4. It can be given for secondary inertia of the uterus, if the patient is not too fatigued. 5. The preferable dosage is 0.5 cc. repeated in 45 to 60 minutes. 6. There seems to be no danger of injury to mother or child. 7. With careful watching, it can be used to give a good trial of labor to borderline Caesarean cases. 8. It does not seem to be effective in inducing labor, hence can be used to differentiate true from false labor.—J. C. D.

Studies on ovulation. II. The effect of hypophysectomy on the formation of the corpus luteum. Deansley, Ruth, A. R. Fee and A. S. Parker, J. Physiol. **70:** 38. 1930.

Hypophysectomy in the rabbit immediately after copulation inhibits ovulation. If performed later inhibition does not result. Control animals were subject to a similar operation. In both groups the development of corpora lutea proceeded in a similar manner but both showed retarded development when compared to unoperated animals.—C. I. R.

A practical test for the antidiuretic action of pituitary. Gibbs, O. S., J. Pharmacol. and Exper. Therap. **40:** 129. 1930.

The author proposes as an antidiuretic unit that amount that will prevent water diuresis in the mouse for three hours.—C. I. R.

Notes on the tissue pH in diabetes insipidus (Recherches sur le pH intratissulaire dans le diabète insipide). Marinesco, G., O. Sager and E. Soru, Compt. rend. Soc. de biol. **103:** 171. 1930.

There is a slight increase of acidity in the tissues accompanying the diabetes. Marked acidity is an indication of secondary conditions producing abnormal metabolism. Four human cases were studied.—J. C. D.

Relations of the endocrine glands to the water content of the blood, muscles, and certain of the endocrine and exocrine glands (Sur les relations des glandes endocrines avec l'hydratation du sang, des muscles et de certaines glandes endo- et exocrines). Parhon, C. I., V. Marza and M. Cahane, Compt. rend. Soc. de biol. **103:** 281. 1930.

A concentrated statement of the authors results. They point out in relation to the physiology of old age that testicular extracts have a water reducing effect; while thyroid extracts, adrenin, extracts of the posterior lobe of the hypophysis, placenta, ovary, thymus, as well as adrenalectomy, increase the water content of various parts simulating the condition found in a young individual.—J. C. D.

On the melanophore cells in the pars intermedia and the lipoidophore cells of the pars posterior of the hypophysis of the pied rat (Sur les cellules mélanophores du lobe intermédiarie et les cellutes lipoïdophores du lobe postérieur de l'hypophyse du rat pie). Parhon, C. I. and Z. Caraman, Compt. rend. Soc. de biol. **103:** 283. 1930.

The authors again direct attention to these peculiar types of cell.—J. C. D

Hypophysis—Midbrain system and fat metabolism (Hypophysen—Zwischenhirnsystem und Fettstoffwechsel). Raab, W., Wien, Klin. Wchnschr. **42**: 238. 1929.

A short survey of the author's previous papers concerning the influence of pituitrin upon fat metabolism: decrease of the level of neutral fat of the blood. This effect is produced by a primary action of pituitrin upon the mesencephalon (tuber cinereum) and a nervous path leading through the cervical spinal cord and the abdominal sympathetic to the liver. The latter absorbs and apparently destroys excess fat amounts of the body under the stimulating influence of the pituitary-mesencephalic system.—Author's Abst.

The small intestine and gastric secretion with special reference to Brunner's glands. Murray, Margaret M., J. Physiol. **69**: 48. 1930.

Extracts from various regions of the duodenum were prepared and their effects on gastric secretion studied. Only those from regions containing Brunner's glands were effective in increasing the amount of gastric secretion, and the amounts of HCl and pepsin.—C. I. R.

A case of diabetes mellitus with thrombo-angiitis obliterans. Adams, S. F., M. Clin. North. America, **14**: 581. 1930.

The case described occurred in a man of 48 who came to the clinic because of a sore of 8 months' duration on the right great toe. The toe was gangrenous and the remaining toes of the right foot were cold and blue and pulsation could not be felt in the dorsalis pedis or posterior tibial artery. Further examination did not suggest the usual features of diabetes gangrene, but proved the patient to be suffering with a combination of diabetes and thrombo-angiitis obliterans. A diebetic diet was given which kept the urine free from sugar without the use of insulin. Typhoid vaccine was given on 5 occasions with the hope of improving the circulation in the feet. The systemic reaction produced by the vaccine did not aggravate the diabetic condition. Postural exercises were advised. The patient was finally dismissed from the clinic in a very much improved condition.—I. B.

Insulin and experimental sores (Insuline et plaies expérimentales). Barral, P. and R. Cade, Compt. rend. Soc. de biol. **103**: 249. 1930.

In white rats there was no difference in the rapidity of healing of a skinned area in those receiving insulin injections and those not.—J. C. D.

Contributions to the chemical study of the blood in experimental pancreatic diabetes in dogs (Contribution a l'étude biochimique du sang dans le diabète pancréatique expérimental du Chien). Cahane, M. and I. Orasteanu, Compt. rend. Soc. de biol. **103**: 277. 1930.

The twelve dogs used showed an acidosis which was compensated for except in cases of coma. Calcium was reduced somewhat, cholesterin markedly increased. The rate of red cell sedimentation was increased considerably.
—J. C. D.

Acute interstitial pancreatitis in two cases of diabetic coma. Foord, A. G. and B. D. Bowen, Am. J. M. Sc. **180**: 676. 1930.

Two cases of acute interstitial pancreatitis, in which the patients died in coma, are reported. In both cases treatment was instituted but with no avail. The authors believe the abdominal pain and leukocytosis present in diabetic coma are not due to acute pancreatitis, but are related in some way to the acidosis.—E. L.

Studies of the action of insulin on tissue cultures (Action de l'insuline étudiée en cultures de tissues). Friedheim, E. and N. Roukhelman, Compt. rend. Soc. de biol. **103**: 10. 1930.

Embryonic mesenchymal cells in vitro when growing in the presence of insulin showed no change in glycogen formation and a reduction in glucose consumption.—J. C. D.

Pancreatic diabetes in hypophysectomised dogs (La diabetes pancreática de los perros hipofisoprivos). Houssay, B. A. and A. Biasotti, Rev. Soc. argent. de biol. **6**: 251. 1930.

Hypophysectomy previous to or after pancreatectomy diminishes the intensity of the diabetes observed (9 experiments in which no infection occured and 5 experiments in which infection did occur). Survival is considerably prolonged and may be as long as 6 months, without injection of insulin. There is a gradual loss of weight, but at times important increases are observed. The blood sugar is usually between 0.13 and 0.323 gm.%, nearly always below 0.3%. In 7 dogs hypoglycemic convulsions were observed; sometimes they were fatal, at others the injection of glucose permitted recovery. Glycosuria was never very marked, on an average 0.059 to 0.89 gm. per kgm. per day, except in 2 cases in which it was 1.3 and 3.23 gm.; fasting diminishes it considerably or even suppresses it. Ingestion of sugar increases the severity of diabetes but only from 30 to 70% of the amount given is eliminated. The D:N ratio is low, 0.70 to 1.86. The basal caloric output was below the normal in 2 cases and slightly above in 1. Lesions of the tuber cinereum do not modify the course of pancreatic diabetes. In dogs with pituitary and pancreas removed degenerative lesions of the parathyroids were observed. All operations were controlled by post-morten examination, those on the pituitary and mid-brain by histological sections.—J. T. Lewis.

Arteriosclerosis in diabetes. Joslin, E. P., Ann. Int. Med. **4**: 54. 1930.

Joslin states that in the present era (last 4 years) every other diabetic (781 fatal cases) dies from arteriosclerosis which is a much higher figure than in previous eras. The most important reason for the rise of arteriosclerosis as a cause of death is in the fall in mortality from coma which has decreased to 11% in the last 781 cases. The prolongation of the life of the diabetic is important as the average age at death is nearing 60 yeasr. In arteriosclerosis the heart is the most frequent site, 19.1%, the legs 13.2%, brain 7.2%, and the kidneys 4.7%. The percentage of deaths from arteriosclerosis increases markedly with the duration of diabetes. Jews are more prone to arteriosclerosis than other races. A small percentage (1.5%) of children have arteriosclerosis. The author guards against gangrene of the feet by frequent examination. He believes that by controlling the diabetic symptoms and allowing a diet approaching normal that diabetics should live as long as average individuals.—E. L.

Diabetes mellitus in pregnancy (Zuckerkrankheit und Schwangerschaft). Nevinny, H. and G. Schretter, Arch. f. Gynäk. **140**: 397. 1930.

The authors describe two cases of diabetic women whose diabetes toward the end of pregnancy became more severe. They bore large babies, both of which died in about thirty days of congenital heart disorder, and one of pneumonia in addition. Blood sugar studies in one child showed 52 mgm.% at birth, rising to 172 mgm.% and maintaining this height until death. In the mothers the blood sugar rose during and after parturition. A lengthy discussion follows of the various factors involved and also of the comparative sugar studies in the maternal, fetal and placental blood and the amniotic fluid.—H. J. J.

Insulin inactivation by human blood cells and plasma in vitro. II. Effect of infection on insulin. Karelitz, S., S. D. Leader and P. Cohen, Arch. Int. Med. **45**: 690. 1930.

Summarizing the results of the various experiments, it was found that human blood plasma and, to a greater degree, human blood cells inhibit insulin action in vitro. Blood from diabetic patients as well as from patients with purulent infections or artificially produced infection-like conditions, such as serum sickness, causes greater insulin inactivation in a given time than does normal blood. Blood cells from a patient with myeloid leukemia and pus also show greater insulin inactivating power than do normal blood cells. This inactivation seems to act better at mildly alkaline p_h and is ineffective at $p_h 6$. After being heated from 55 to 60° C. from one to two hours or after incubation at icebox temperature, it also becomes ineffective. The inactivation requires time and is not present when the blood-insulin mixture is injected

directly into the blood stream. The occurrence of this inactivating substance and its properties as described are so similar to the occurrence and action of enzymes that it may be that an enzyme, possibly trypsin, or an enzyme-like substance is responsible for the insulin inactivation. The observations being limited to the effect of blood outside of the human body on insulin action, it cannot be certain that this phenomenon occurs within the living organism. It is, however, conceivable that when cells are injured as they are during infection, an abnormal state comparable to in vitro conditions exists, enabling this substance to act on insulin. Perhaps in these states of abnormal metabolism, as during infection, inadequate insulin production, neutralization of insulin by action of thyroid, suprarenal, pituitary, and other ductless glands secretions, as well as altered enzyme action throughout the body or in certain locations, are all responsible, acting simultaneously and in varying degrees.
—Authors' Summary.

Mild diabetes and severe diabetes. Patmos, M., E. C. Bartels and S. F. Adams, M. Clin. North America, **14**: 799. 1930.

This is a good though brief description of the various forms of diabetes with a discussion on a series of cases. Diabetes is a disease of varying severity. The authors emphasize that diabetes of any degree is to be regarded as an ever-present threat. In 3 of 27 cases of mild diabetes and in 7 of 72 cases of severe diabetes coma developed. It is significant that the incidence of coma in the cases of mild diabetes and in the cases of severe diabetes is about the same. It appears that the incidence of severe diabetes is about equal among men and women. There were twice as many cases of mild diabetes in men as in women.—I. B.

On oral insulin therapy (Ueber perorale Insulinwirkung und perorale Insulintherapie). Stephan, R., München. med. Wchnschr. **76**: 1579. 1929.

Experimental investigations concerning the activity of insulin given by mouth showed positive effects when dry insulin was applied in oily or fatty mixtures during emptiness of the stomach and after regurgitation of bile from the duodenum. X-ray controls showed that the preparations were effective only during absorption from the stomach but not from the intestins. The best results were obtained with a preparation which is a compound of desoxycholic acid and insulin. The influence upon the blood sugar level is to a high degree independent of the dosage and the shape of the blood sugar curves is rather irregular. The depressant effect lasts longer than after subcutaneous application; the lowest point is sometimes reached within 10 hours. The effect in diabetics is particularly conspicuous after the intake of food; thus the alimentary hyperglycemia is checked by the preparation. It has been tried in 12 cases for therapeutical purposes with fairly satisfactory result.
—W. Raab.

Epilepsy vs. diabetes. Trumper, M., Pennsylvania Med. J. **34**: 18. 1930.

This is a brief study of the subject from the viewpoint of the biochemical laboratory technician. In epilepsy and diabetes the fundamental mechanisms are, in part at least, antagonistic. Diabetes is associated with ketosis and anhydremia; epilepsy on the contrary is characterized by ketosis and hydremia and is benefited by therapy designed to produce a state of ketosis and dehydration. Theoretically therefore the incidence of epilepsy in diabetes should be extremely rare. On the other hand one would not expect diabetes to be a particularly unusual complication of untreated epilepsy. Clinical experience confirms the theoretic observation to the effect that the treatment of epilepsy by means of the ketogenic diet involving a diminished carbohydrate intake diminishes the likelihood of the development of diabetes in a predisposed individual. The author quotes Joslin who states that he is unable to find a clearcut case of epilepsy among 5086 true diabetics. If an epileptic is known to have diabetes, care must be exercised in the use of the ketogenic diet for the control of the epilepsy since the deliberate production of a ketosis superimposed upon the already existing state of ketosis which accompanies diabetes may have serious consequences.—I. B.

Etiologic factors in diabetes mellitus when its first manifestations follow exogenic influences (Grundsaetzliches zu der Frage der aetiologischen Begutachtung des diabetes mellitus nach seinem ersten auftreten im anschluss an exogene Einwurkungen). Veil, W. H., Deutsche med. Wchnschr. **56:** 729. 1930.

A long discussion as to whether trauma can cause diabetes. The case is that of a boy ten years old, who was an excellent gymnast. While training hard in the heat of summer he developed an acute onset of diabetes. Two years previously the urine was sugar free.—H. J. J.

Contributions to the study of the physiological variations in the internal secretion of the pancreas. VIII. Reaction of the external activity of the pancreas on the secretion of insulin (Contributions a l'étude des variations physiologiques de la sécrétion interne du pancreas). Zunz, E. and J. La Barre, Arch. internat. Physiol. **33:** 142. 1930.

The introduction of dilute HCl into the duodenum of a dog lowers the blood sugar. The lowering of blood sugar is due to an increase of the insulin secretion. This was proved by using the transfusion method developed by these investigators. This phenomenon does not depend on the vagus because it will take place after section of the vagus. The introduction of physiological salt solution, into the duodenum has no effect on blood sugar.—E. L.

Hyperparathyroidism: with report of a case. Pemberton, J. de J. and K. B. Geddie, Ann. Surg. **92:** 202. 1930.

The case reported by Pemberton and Geddie is the seventh proved case of hyperparathyroidism and the sixth in which the condition was due to tumor. These cases record a remarkably complete and relatively new clinical syndrome. Based on a knowledge of the physiology of the parathyroid glands, as recently demonstrated by numerous observers, these cases represent exactly what one would expect if the body were subjected to an excess of the parathyroid secretion over a relatively long period. In one case, the criteria on which the diagnosis of hyperparathyroidism was made is as follows: low serum calcium; high serum phosphorus; cataract; normal density of bones, and aggravation of tetany by exertion. All of the cases referred to in this article demonstrated exactly converse changes. The patient reported by Pemberton and Geddie was a girl, aged fourteen years, whose chief compaint was spells of vomiting, pallor and loss of weight. Her weight had decreased and she had become constipated and had polydipsia and polyuria; she drank about two quarts of water each night. The patient appeared pale and emaciated. Complete gastro-intestinal examination did not reveal organic lesions. The blood count revealed moderate secondary anemia. The urine usually contained a trace of albumin and occasionally a few pus cells. Roentgenograms of the bones showed diffuse decalcification. In view of the similarity of this case to several of the other cases of hyperparathyroidism, a tentative diagnosis of parathyroid tumor was made, and operation was performed. Lying behind the left lobe of the thyroid gland at the inferior pole and outside of the capsule but attached thereto, was a tumor, 1.5, 1.25 and 1.25 cm. in various diameters. Following the operation the gastro-intestinal symptom cleared up. The return of renal function to normal has not been so prompt as in some of the other cases. Six days after operation the excretion of phenolsulphonephthalein was the same as before. The water concentration tests revealed slight improvement in the ability of the kidneys to concentrate fluids. Electrical reaction was not markedly lacking before operation, but it was practically normal a few days after operation. Roentgenograms of bones four weeks after operation did not reveal increase in density. There are no means of knowing to what extent the process in the bones would have gone had the parathyroid tumor not been removed.—J. de J. Pemberton.

Studies on the inorganic constituents of the blood of normal and parathyroidectomized dogs. Weaver, W. K. and C. I. Reed, J. Biol. Chem. **85:** 281. 1929.

Simultaneous determinations of calcium, inorganic phosphorus, sodium, potassium, and magnesium were made in 16 dogs over preoperative periods ranging from 2 weeks to 3 months and from 3 days to 6 weeks after thy-

roparathyroidectomy. No constant changes were noted in the concentration of any of these substances except calcium and inorganic phosphorus. The production of latent tentany does not necessarily depend on stimulation of accessory parathyroid tissue since this condition may readily occur without restoration of calcium and phosphorus to normal proportions.—C. I. R.

The thymus and tar cancer (Thymus et cancer du goudron). Babes, A., Compt. rend. Soc. de biol. **103**: 165. 1930.

Six rabbits, four cancerous and two controls, were examined. The thymus was large in the controls and small in the injected animals. The author suggests that the small size of the thymus may have been a predisposing factor since all six animals were exposed to tar treatments.—J. C. D.

Diagnosis and treatment of thymoma. Craver, L. F., M. Clin. North America, **14**: 507. 1930.

The diagnosis of thymoma may be made with fair assurance during the patient's life in a majority of cases, on the basis of symptoms, physical signs, and roentgenographic findings. The treatment should be irradiation with high-voltage roentgen rays or the radium or radium emanation pack. Cases of thymoma should be closely observed at frequent intervals indefinitely, since metastases or extensions of the tumor may give rise to many unusual complications.—I. B.

A co-occurrence of enlarged thymus and congenital hernia. Edwards, E. A., New England J. Med. **203**: 969. 1930.

In Boston City Hospital over a period of three years, twenty-six cases of enlarged thymus among about 2500 patients under 16 years were observed. Of these, 38% had congenital hernia. The author considers that this may be coincidence or association.—J. C. D.

Pathological thymus in children from a roentgenological standpoint. Gilmore, W. H., Illinois J. **58**: 97. 1930.

This is a roentgenologist's views on enlarged thymus in children and the clinical implications to be derived therefrom. Cases in which a careful x-ray study shows enlargement of the thymus should be treated irrespective of the presence or absence of physical findings. Any shadow in the upper anterior part of the chest in children under 8 years of age which cannot be accounted for by the shadows of the great vessels, should be looked upon with suspicion, particularly when the physical condition is below normal. It is often rather difficult to take satisfactory films in children under 3 years of age because of lack of the patient's cooperation, so that the services of 3 or 4 adults may be necessary in this connection. The treatment of enlarged thymus in children is discussed.—I. B.

Diagnosis and treatment of diseases of the thymus. Johnson, J. B., South. M. J. **23**: 755. 1930.

No ill effects follow x-ray of the thymus, so that all children showing enlarged glands or showing possible thymic symptoms should receive the benefit of this treatment.—J. C. D.

Thymus. Martin, D. D., South. M. J. **23**: 625. 1930.

A review warning against the x-ray treatment of enlarged thymus as a routine measure and pleading for a study of thymic enlargement in reaction to other disorders.—J. C. D.

Thyro-intoxication with necrosis and atrophy of the liver, damage to the heart muscle and terminal bronchopneumonia. Barker, L. F., M. Clin. North America, **14**: 261. 1930.

This illustrates another of the cases on record of atrophy of the liver in association with Graves' disease. The patient described was a white woman of 64 who died the day after she was admitted to the hospital in a state of

coma. Post mortem findings were thyroid hyperplasia, large heart with myocardial insufficiency, pulmonary emphysemia with beginning bronchopneumonia, small liver containing areas of necrosis and of passive congestion secondary to cardiac failure, tubular degeneration of the kidneys, and carcinoma of the cervix.—I. B.

Cholesterin in dogs. Influence of hyperthyroidism and castration (Cholestérinémie chez le Chien. Influence de l'hyperthyroïdie et de la castration). Blinoff, A., Compt. rend, Soc. de biol. **103:** 188. 1930.

Four dogs were observed; a control, a castrate, one fed with thyroid, and one castrated and fed with thyroid. Thyroid feeding reduced the cholesterin in both cases. Castration alone produced a slight increase.—J. C. D.

The thyroid-hypertrophy-subnutrition syndrome. Bram, I., Illinois M. J. **57:** 400. 1930.

In what is termed the thyroid-hypertrophy-subnutrition syndrome a condition is described occurring in young women varying in age between 19 and 30, characterized essentially by thyroid hypertrophy of average degree, underweight, secondary anemia, nervousness, weakness, occasional palpitation, some shortness in breath, and general inefficiency. While thyroid swelling, underweight, and cardiac irritability may lead to a suspicion of hyperthyroidism, the latter is excluded by the absence of a heightened metabolic rate. On the contrary, many of these patients, despite nervousness and heart hurry, present a metabolic rate of minus 10 to minus 20%. The thyroid swelling is not causative of the symptomatology, but rather the result of the undernourishment. The etiology resides in erroneous habits of life, particularly in the quality and quantity of diet, sleep, work, play, and even the thought processes. In the event of faithful cooperation the prognosis in these cases is excellent. Education of the individual to the need for a correction of life's habits, particularly the taking of ample quantities of wholesome, non-irritating food, ample sound sleep under hygienic conditions, and the living of a simpe life usually results in cure within a few months. Such remedies as eserine to overcome evidences of sympatheticotonia, iron, arsenic, cod liver oil and a mild sedative are useful adjuvants in treatment.—Author's Abst.

The diagnosis of borderline hyperthyroidism. Clute, H. M. and H. D. Adams, New England J. Med. **202:** 1135. 1930.

Of 69 patients on whom a diagnosis of borderline hyperthyroidism was made and who were subjected to thyroidectomy, 58 were freed of their symptoms. In 11 patients no improvement followed operation. Analysis of the 11 failures shows that in each case probably no hyperthyroidism was present at the time of operation. Persisting myxedema occurred in 6 cases, but with treatment these patients are free from troublesome symptoms. When hyperthyroidism is definitely proven in borderline cases, thyroidectomy is indicated and will be of marked benefit. In questionable hyperthyroidism, no thyroidectomy should be undertaken until long observation renders the diagnosis positive.—J. C. D.

The comparative effect of different iodine preparations in the preoperative treatment of thyrotoxicosis. Fulton, M. N. and H. L. Alt, New England J. Med. **203:** 327. 1930.

This study based on forty-two bed patients under hospital conditions showed that Lugol's solution, calcium iodobelienate (sajodin) and saturated potassium iodide were effective in the usual therapeutic doses. Sajodin reduces the basal metabolism somewhat less than the other preparations but acts more quickly, is tasteless, and does not produce unpleasant gastric symptoms.—J. C. D.

Remote symptoms of unrecognized thyroid adenomata. Gilman, P. K. and W. E. Kay, Am. J. M. Sc. **180:** 405. 1930.

The authors report thirteen cases in which nervousness was the most frequent symptom though it was not the type usually met in thyrotoxicosis. Weakness and fatigibility were also common. The basal metabolic rate was

below normal in three of the five cases presented in which adenomata were found. All of the patients were benefited by surgical removal of the adenomata.
—E. L.

The problem of thyroid intoxication. Graham, R. R., Canad. M. A. J. **23**: 349. 1930.

Thyroid intoxication is a metabolic disease involving the whole organism, the thyroid dysfunction playing a predominant part. Iodine should never be used in thyroid intoxication except during the pre- and post-operative management. It never cures hyperthyroidism. In frank thyroid intoxication radical resection of the thyroid gland is the therapy of choice and will remain so until we have more accurate knowledge of the underlying etiological factors. Patients suffering from a cardiovascular disability and a nodular thyroid should have a resection of the thyroid even if the basal metabolic rate be normal. Thyrotoxic patients under 20 years of age present a difficult clinical problem. If the symptoms develop with a persistent adolescent enlargement of the thyroid the prognosis is doubtful as far as freedom from morbidity is concerned. Radiation is useful in this group. Operation is attended with much greater risk than in older patients and should only be considered when rest and drugs with removal of all foci of infection have proved inefficient. If thyroid intoxication occurs during pregnancy, the hyperthyroidism should be treated without considering the pregnancy. All nodules in the thyroid are potentially dangerous, and, if persisting after 25 years of age, should be resected. A pre-operative preparation characterized by most minute detail is essential to the successful management of thyrotoxic patients and a prolonged, carefully supervised convalescence is essential to secure the maximum improvement from treatment.
—A. T. C.

The treatment of diseases of the thyroid gland. Guion, C. M., M. Clin. North America, **14**: 375. 1930.

A general review of the common forms of thyroid disease and suggestions on their treatment.—I. B.

The adrenalin test and cholesterol determination in the diagnosis of borderline hyperthyroidism. Hinton, J. W., Am. J. M. Sc. **180**: 681. 1930.

A brief resume of the clinical and laboratory findings in 26 cases of hyperthyroidism is reported. In practically all cases, the adrenalin test of Goetsch was positive. There was no difference in degree of reaction of the adrenalin test which would aid in the diagnosis. The cholesterol findings were not significant.—E. L.

The relation of endemic goiter to mental deficiency. Kimball, O. P. and C. J. Marinus, Ann. Int. Med. **4**: 569. 1930.

From preliminary work in Michigan and Ohio, the authors were impressed with the relationship between mental retardation and goiter. In this investigation, 3548 mentally subnormal children were examined. Of this number 712 showed some endocrine dysfunction and 281 of these showed the result of congenital hypothyroidism. Of the 205 which had had repeated intelligence tests, 90 had a normal rate of growth but their intelligence quotient (I. Q.) was below normal. In 117 cases the I. Q. was decreasing which is due to the fact that the mental age had reached its height whereas the chronological age was increasing. Cretins are predestined to a low mental age which seldom reaches above six years if untreated. If treated, the mental age will be approximately eight years. This fixed low mentality is the result of maternal hypothyroidism. The authors are convinced that the cretinoid type of mental growth is due to endemic goiter. The prevention of goiter as carried out in the state of Michigan has reduced goiter in the Detroit public schools from 42% in 1924 to 7% in 1929. The 7% nearly all have congenital goiter.—E. L.

The temperature reaction following subtotal thyroidectomy. Lerman, J., New England J. Med. **203**: 254. 1930.

One hundred and seventy cases were studied. The author concludes that the degree and duration of the temperature reaction following subtotal thyroidectomy for exophthalmic goitre does not bear any relationship to the

severity of the disease as judged from the average initial metabolic rate. This reaction is not influenced by the degree of fever present before the operation. A slight elevation of temperature, even though it is associated with a mild, protracted upper respiratory infection, is no contra-indication to operation. The maximum height of the post operative temperature reaction does not vary with the season of the year, but the duration of the reaction is definitely longer in the late winter and early spring than in the late summer and early fall. —J. C. D.

Unrecognized hyperthyroidism masked as heart disease. Levine, S. A., Ann. Int. Med. **4**: 67. 1930.

The author presents 8 cases in which symptoms of heart disease were present but were not ordinary cases of exophthalmic goiter or toxic adenoma. These obscure cases were diagnosed by the general appearance of the patient and the history. These patients have a moist, warm skin, prefer cold to warm weather and have short periods of frequency of bowel movement and vomiting. The most characteristic heart findings is transient auricular fibrillation. The heart rate when hyperthyroidism is present will rarely be below 100 on full doses of digitalis. The basal metabolism tests will be above normal. The patient should be kept in bed and iodine therapy instituted. After 10-14 days of treatment a one-stage subtotal thyroidectomy should be performed. In all cases, complete cure or marked improvement was obtained.—E. L.

Studies on the etiology of goiter including Graves' disease. Marine, D., Ann. Int. Med. **4**: 423. 1930.

A brief history of goiter serves as an introduction to a review of present developments in this field. Marine is convinced that the fundamental disturbance in Graves' disease lies elsewhere than in the thyroid gland and is due to a loss of control of the oxidative processes. Marine believes that the following discoveries mark an advance toward the solution of Graves' disease. (1) The symptom complex produced by injury to the suprarenal glands which simulates Graves' disease. (2) The discovery of Szent-Gyorgyi of an active reducing substance from the suprarenal cortex. (3) Glycerol extracts of suprarenal cortex which cause a lowering of metabolism. Though there has been an enormous amount of work done in an effort to prove the infections etiology of Graves' disease, nothing has been proven. The relative or absolute deficiency of iodine resulting in simple goiter may be due to several factors. A low iodine intake, a poor utilization or absorption of iodine or a temporary increase in the need of the organism, for thyroxin may lead to a compensatory hypertrophy of the thyroid. Rabbits fed mainly on cabbage maturing in late autumn will have goiters. The author has been able to separate the goitrogenic substance from winter cabbage and an anti-goitrogenic substance from summer cabbage.—E. L.

The sulphur metabolism in hyperthyroid animals (La thiémie chez les animaux hyperthyroïdisés). Parhon, C. I. and M. Cahane, Compt. rend. Soc. de biol. **103**: 191. 1930.

There is an increase of sulphur at first, with later a diminution to normal limits, in dogs; no changes were found in rabbits.—J. C. D.

Mineral and lipoidal phosphorous of the blood in hyperthyroid animals (Sur le phosphore sanguin salin et lipoïdique chez les animaux hyperthyroïdisés). Parhon, C. I. and M. Cahane, Compt. rend. Soc. de biol. **103**: 284. 1930.

In five rabbits and two dogs fed with thyroid, there was a decrease in the phosphorous salts and an increase in the lipoidal form.—J. C. D.

The treatment of exophthalmic goiter (Le traitement du goitre exophthalmique). Pinto, A. and Coelho, E., Presse méd. **38**: 673. 1930.

This is a review of the surgical treatment of exophthalmic goiter, with a discourse on the preoperative and postoperative administration of iodine. Instead of Lugol's solution preoperatively, the authors employ Neisser's solution beginning with 6 drops daily and increasing to a maximum of 30 drops daily

during a period of 3 weeks. Occasionally digitalis and quinidine are also employed for the amelioration of outstanding symptoms of the disease. The authors favor radical resection of the thyroid gland. Five illustrative case histories are included.—I. B.

The significance of acidophilic cells in the thyroid gland. Warren, S. and W. Stoller, New England J. Med. **203**: 523. 1930.

Groups of acidophilic cells were encountered in 75 cases. Thirteen of these later developed post-operative myxedema. The presence of of these cells is not of practical significance in foretelling which patients will become myxedematous.—J. C. D.

The effect of thyroparathyroidectomy on experimental gastric defects. Winkelstein, A. and J. M. Ziegler, J. Lab. & Clin. Med. **16**: 1. 1930.

Patients with chronic gastric or duodenal ulcer present a hyperirritability somewhat suggestive of latent tetany. To investigate a possible relationship, a parathyroidectomy was performed on 60 dogs and 4 cats. Twenty dogs and 3 cats died in tetany without the administration of parathyroid extract. Twenty-four dogs dying in tetany had received parathyroid extract for 2 weeks only. Nine dogs, after 2 weeks of parathyroid extract administration, lived normally without it, and in these, an ulcer excised in the stomach at the incisura angularis was found healed in 10, 38, 49, 53, 57, 34, 48, 77, and 92 days respectively. The authors conclude that a circular gastric defect, 5 cm. in diameter, extending down to the serosa in the lesser curvature of the incisura angularis, heals completely in approximatey 1 to 2 months in an animal in whom a thyroparathyroidectomy has previously been performed. Apparently neither a thyroid insufficiency nor a latent tetany interferes with the healing of a gastric lesion in the dog or cat.—I. B.

Thyroidectomy and experimental diabetes (Tiroidectomía y diabetes experimental). Yriart, M., Rev. Soc. argent. biol. **6**: 297. 1930.

In dogs the thyroid was removed aseptically leaving the parathyroids. Animals free from symptoms of tetany had their pancreas totally removed 10 to 20 days later. The onset of diabetes was delayed by insulin treatment till they were healed, then insulin was discontinued. Diabetes had the same severity as in controls with thyroids intact as revealed by glycosuria, blood-sugar level, D:N ratio, body weight and time of survival. Partially pancreatectomised dogs with a progressive diabetes (Sandmeyer) were thyroidectomised, the evolution of the disease was not affected. Blood-sugar curves following intravenous injection of 1 gm. glucose per kgm. were identical before and after thyroidectomy. Dogs fed with thyroid powder until toxic symptoms appeared showed no alteration in the blood sugar curve following glucose injection. Partially pancreatectomised dogs showed a slight increase in severity of diabetes when fed with thyroid.—J. T. Lewis.